Eastern Massasauga Rattlesnake: Evaluating the Effectiveness of Mitigation Structures at the Population Level

by

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Abstract

As reptile populations worldwide decline, construction and monitoring of road mortality mitigation measures is becoming common. I studied the effectiveness of barrier fencing at preventing Eastern Massasauga rattlesnakes (*Sistrurus catenatus*) from gaining access to the road. I also tested whether ecopassages were effective at allowing Massasaugas to access habitat on both sides of the road. I determined that there was a reduction of Massasaugas on the road post-installation of barrier fencing. Data from various monitoring approaches showed that Massasaugas do indeed use the ecopassages to cross the road. I quantified the long-term effect of mitigation structures on the population viability of Massasaugas. A Population Viability Analysis revealed that post-mitigation construction, the study population has a low probability of extinction, suggesting that mitigation is effective at promoting a sustainable population. Analyzing the effects of road mortality at the population level is crucial to ensure that decision makers are adequately informed of the status of species-at-risk.

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General Introduction

Scientists are increasingly concerned over the dramatic and accelerating loss of biodiversity worldwide (Blaustein & Kiesecker 2002; Böhm et al. 2013). It is difficult to quantify the exact rate of species disappearance, but it is estimated that it is at its highest in recent history, and has thus been dubbed the "sixth mass extinction" (Eldredge 1998). These worrying trends are attributed to a variety of causes such as habitat fragmentation, habitat loss, climate change, persecution by humans, and other human disturbances. Globally, habitat loss from human population growth and associated activities is a leading cause of extinction (Diamond et al. 1989; Gibbons 2000; Brooks et al. 2002) and is further exacerbated by habitat fragmentation, which is known to impede animal dispersal and gene flow (Eigenbrod et al. 2008; Shepard et al. 2008; Leidner & Haddad 2011) and restrict wildlife movement across the landscape (Ashley & Robinson 1996; Gibbs & Shriver 2002; Rouse et al. 2011; Brehme et al. 2013).

Transportation Corridors:

Today's landscape presents an array of evidence for one of the most widespread anthropogenic changes in the past century: the creation of transportation corridors such as railways, roads, and major highways coincident with the rise of automobiles (Bennett 1991; Reed et al. 1996). Roads have major consequences for wildlife habitat and are considered a threat to the survival of many populations and even entire species (Ashley & Robinson 1996; Trombulack & Frissel 2000; Jaeger et al. 2005, 2006; Clark et al. 2010; Rouse et al. 2011). In North America, approximately 15-20% of the total land area is impacted by roads (Reijen et al. 1995), and, in Ontario alone, the primary road network has increased from 7 000 km total length in 1935 to 350 000 km in 1995 and traffic volume on Ontario roads has been exponentially increasing over the last 30 years (Fenech et al. 2001; OMTO 2010). As human populations grow, traffic volumes increase, wherein road networks continue to expand creating an endless feedback loop (Forman & Alexander 1998). Direct road mortality is a well-known impact of roads on animal populations (Santos et al. 2001; Jaeger et al. 2005; Row et al. 2007; Rouse et al. 2011; Baxter-Gilbert et al. 2015), but ecological damage is not limited directly to the roadway itself.

Society has grown to depend on transportation networks for the trade of products and the travel of people across distances (Andrews et al. 2008). Studying the ecological consequences of continued development of transportation infrastructure is a main focus of research for many biologists, leading to the relatively new field of study, road ecology. Mitigation measures to alleviate the negative effects of roads on the environment and individual species will be an important future direction for both research and implementation by planners and biologists (Mader 1984; Rosen & Lowe 1994). My thesis contributes to this emerging body of research by thoroughly evaluating mitigation structures to reduce snake mortality within a single Canadian locale, Killbear Provincial Park.

The Rise of Road Ecology:

Roads can have negative impacts on the long-term viability of wildlife populations (Andrews et al. 2008). One such impact is the creation of population sinks through direct mortality from vehicular traffic, where sustained mortality within one locale results in a reduction of the overall population over time. Population sinks potentially limit wildlife populations and reduce species richness and abundance (Fahrig et al. 1995; Aresco 2005; Glista et al. 2009). There is also evidence that transportation corridors can restrict the movement of wildlife across the landscape (Ashley & Robinson 1996; Gibbs & Shriver 2002; Rouse et al. 2011; Brehme et al. 2013) contributing to population fragmentation and isolation.

As road networks are developed and expanded, the need to understand the various ways that roads affect ecosystems is increasing. Trombulak and Frissell (2000) identified seven ways that roads can affect the natural environment: 1. increased mortality from road construction, 2. increased direct mortality from collision with vehicles, 3. modification of animal behaviour, 4. alteration of the physical environment, 5. alteration of the chemical environment, 6. spread of exotic and invasive species, and 7. increased alteration and use of habitat by humans. My study primarily assesses the effects of direct mortality.

Habitat loss is not only caused by the construction of roads themselves, but also through the fragmentation of the landscape (Trombulack & Frissel 2000; Lesbarrères & Fahrig 2012). Roads create impediments to movement to varying degrees depending on the life history of organisms in question, and can thus isolate previously contiguous populations (Trombulack & Frissel 2000). Animals subject themselves to dangerous conditions on roads simply to access resources found in the habitats on the alternate sides of the road, resources on the road itself or to disperse (Mader 1984; Andrews, 1990). Urbanization can exacerbate the situation, making roads essentially impassable for particular wildlife species, negatively effecting the distribution of populations and resulting in reduced genetic connectivity (Vos & Chardon, 1998; Epps et al. 2005; Riley et al. 2006; Richardson et al. 2006; Row et al. 2007; Dileo et al. 2013).

Effects of Roads on Herpetofauna:

Reptiles and amphibians are suffering among the largest declines of all animal groups due to the effects of roads (Fahrig et al. 1995, Ashley & Robinson 1996; Jochimsen et al. 2004). Reptiles and amphibians are particularly vulnerable to environmental changes, including the construction of new transportation infrastructure, due to key physiological and behavioural traits like basking (Andrews et al. 2008). For several species, their vulnerability to the consequences of road mortality is exacerbated by their life histories that include longevity, late sexual maturity and low reproductive rates (Haxton 2000; Enge and Wood 2002). These life history traits mean that even minimal adult mortality can result in devastating effects on their populations (Congdon et al. 1994; Blouin-Demers & Weatherhead 2002).

While road mortality is a threat to all herpetofauna, threats to snakes are of particular concern (Kjoss and Litvaitis 2001; Roe et al. 2006; Mullin & Seigel 2009). Snakes are especially vulnerable to road mortality during their seasonal movements to and from hibernation, gestation and breeding sites (Pope et al. 2000). For example, Massasauga Rattlesnakes (*Sistrurus catenatus*) in Missouri suffered greater road mortality while returning to their hibernacula from their summer ranges compared to the rest of the surveying season (Seigel 1986). Compared to most other wildlife, the slow movement of snakes makes them particularly prone to direct road mortality (Andrews & Gibbons 2005). Some snakes are also more susceptible to being run over by vehicles as they display immobilization behaviour to oncoming vehicles; this resembles a reaction to predators and has been especially noted in rattlesnakes (Andrews & Gibbons 2005). In addition, snakes are attracted to the road surface for thermoregulation, prolonging their time on the road and furthering their exposure to vehicular mortality (Dodd et al. 1989, Rosen & Lowe 1994; Enge & Wood 2002). Alongside the threat of direct mortality, roads act as a barrier to snake movement that results in less available habitat, posing a risk to population viability (Sheppard et al. 2008; Rouse et al. 2011).

Secondary threats, like the illegal pet trade, can be augmented by roads (Andrews et al. 2008). Road creation and upgrade increases the accessibility of humans to snake habitat, which in turn may lead to increased poaching, harassment, and persecution (Trombulack & Frissel 2000). Roads also increase the openness of an area, causing snakes to be more visible to predators as they utilize the roadway for crossing or basking (Andrews et al. 2008). For example, hawks have been observed predating on crossing rattlesnakes (Vandermast 1999).

There are many complicating factors that can increase the intensity of ecological damage caused by roads. Vehicle speed, traffic density, local topography, surrounding landscape, structural condition of the road, and type of road can all affect the number of species killed (Clevenger et al. 2003). Road mortality can be exacerbated when daily or seasonal activity patterns of wildlife overlap times of high traffic volume (Rosen & Lowe 1994, Hels & Buchwald 2001). Although often overshadowed by direct mortality from traffic collisions, the construction of roadways can cause declines in reptile populations indirectly via destruction of habitat (Weatherhead & Prior 1992).

Recent studies have helped to fill the knowledge gap on how road mortality has negatively affected herpetofauna, and the resulting potential loss of some populations and species (Aresco 2005; Row et al. 2007; Andrews et al. 2008; Baxter-Gilbert et al. 2015). My thesis quantitatively assesses the impact of road mortality on the demographics of a population of one snake species at risk, augmenting knowledge on long-term population level effects of direct road mortality.

Wildlife mortality, including road mortality can be considered additive or compensatory (Litvaitis & Tash 2008; Sandercock et al. 2011). Additive road mortality is defined as a decline in annual survival as a result of increased road mortality (Litvaitis & Tash 2008; Sandercock et al. 2011). Compensatory road mortality considers mortalities occurring as a replacement form of mortality (Litvaitis & Tash 2008; Sandercock et al. 2011). Studies have tried to address additive versus compensatory mortality in road ecology but studies of this nature are very complex. (Huijser & Bergers 2000; Litvaitis & Tash 2008). Although compensatory road mortality is important to consider, due to the constraints of my study, I will focus on additive road mortality.

Mitigating the Effects of Roadways:

Various methods have been suggested to mitigate the damage caused by roadways; a solution commonly employed is the construction of crossing structures with connecting barriers to direct wildlife movement (Andrews et al. 2008). The goal of crossing structures, such as culverts, is to allow safe passage of wildlife across roads and to re-connect the habitat that the roads originally bisected (Forman et al. 2003). Barrier fencing is installed alongside a roadway to channel wildlife towards the crossing structure. Barrier walls and culvert systems have been used to reduce wildlife road mortality (Dodd et al. 2004), but their success varies among locations (Yanes et al. 1995; Baxter-Gilbert et al. 2015). Many factors determine the effectiveness of crossing structures including size, shape (Cain et al. 2003; Clevenger & Waltho 2005), moisture, temperature, substrate (Beier 1995) and location (Yanes et al. 1995; Foster & Humphrey 1995). As these structures can be costly, economic factors are usually one of the largest concerns when planning these mitigation measures (Mata et al. 2008; Huijser et al. 2009; Glista et al. 2009).

Regional declines of reptiles will continue unless effective mitigation measures are found to reduce mortality and maintain population connectivity across road networks (Compton et al. 2007). Although much has been learned about the effects of roads on reptiles and possible mitigation measures, we lack detailed follow-up studies to assess the effects of barrier fences and ecopassages on reptiles (Dodd et al. 2004; Lesbarrères & Fahrig 2012; Baxter-Gilbert et al. 2015). To minimize negative effects caused by roads, we need to further understand whether barrier structures enhance connectivity and diminish mortality in reptile species (Andrews et al. 2008).

Massasauga Rattlesnake:

The Eastern Massasauga (*Sistrurus catenatus*) occupies four regions in Ontario: the Ojibway Prairie Complex near the city of Windsor, the Wainfleet Bog near Port Colborne, the northern half of the Bruce Peninsula and eastern shores of Georgian Bay, including Killbear Provincial Park (Parks Canada Agency 2013). The Massasauga was historically more widespread throughout Ontario, but has lost an estimated half of its historical range within the past two centuries due to habitat loss and persecution (Parks Canada Agency 2013). Road mortality is one of the main factors causing the decline of the Massasauga (Parks Canada Agency 2013) and has the potential to destroy local populations and limit movement between critical habitats. My thesis focuses on assessing the efficacy of barrier fences and four ecopassages that were installed to reduce road mortality in Killbear Provincial Park from 2007-2013. These mitigation measures were constructed to both reduce mortality rates of Massasaugas and aid in the long-term survival of this well-known local population. In Chapter 1, I evaluate the efficacy of the fences and ecopassages by directly analyzing the ability of the fencing to prevent access to the road, thereby reducing road mortality, and the ability of the ecopassages to enhance population connectivity. Chapter 2 includes an assessment of the population viability of Massasaugas in Killbear to determine the status of the current population and whether current mitigation will result in a sustainable long-term population.

Significance

The overall goal of my thesis was to quantify the effectiveness of mitigation structures in reducing Massasauga road mortality. Such assessment of road threats to reptiles is imperative as additional mortality to populations may drastically diminish long-term population viability. The evaluation of mitigation structures at the population level is essential to truly define success. Ultimately knowledge of successful mitigation techniques is crucial to the proper management of species at risk and the development of recovery plans. At a time when funding is limited, it is important that mitigation methods undergo rigorous evaluations to ensure valuable funds are spent most effectively.

My project will inform future design and construction of ecopassages in other key road mortality locations for snakes. This information will contribute to a suite of literature on mitigation techniques that can be used for proposed road developments in critical reptile habitat. The lessons learned from evaluating mitigation techniques could prove to be beneficial additions to the field of road ecology and ultimately assist in mitigating wildlife-road conflicts in future road developments. As we understand more about mitigation, we have a better chance at reducing road mortality and recovering populations.

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Chapter 1

Killbear Story:

An evaluation of the effectiveness of mitigation for reducing road mortality of Eastern Massasauga Rattlesnakes

Abstract

Reducing road mortality is essential to reptile conservation. The eastern Georgian Bay, Ontario regional population of the Eastern Massasauga rattlesnake (*Sistrurus catenatus*) is designated as Threatened by COSEWIC, in part because of high road mortality. Killbear Provincial Park has taken steps to reduce reptile road mortality through construction of four ecopassages and barrier fencing along three busy park roads. Although mitigation of road mortality has been widely recommended for reptiles, its effectiveness has rarely been evaluated. To address this deficit, I monitored park roads twice daily on bicycle, and again at night by car to document locations of both living and dead Massasaugas and other reptile species in 2013 and 2014. Road mortality data were compared pre-, during and post-mitigation. Automated PIT tag readers and trail cameras were installed at each ecopassage to monitor snake activity. A “willingness to utilize” (WTU) experiment was conducted to further explore the effectiveness of the ecopassages. I found a significant decrease in road mortality of Massasaugas on park roads over time as mitigation was constructed. Monitoring techniques showed that Massasaugas and other reptiles used the ecopassages, observations that were further supported by the WTU experiment. My evaluation of mitigation structures determined that they are successful at reducing road mortality and connecting bisected habitats, provided that intense maintenance of the fencing is conducted yearly. My study provides a template for construction of similar mitigation in other key locations where reptile road mortality occurs.

Introduction

Roads have major consequences for wildlife habitat and are considered a threat to the survival of many species (Ashley & Robinson 1996; Trombulack & Frissel 2000; Jaeger et al. 2005, 2006; Clark et al. 2010; Rouse et al. 2011). Impacts from roads can affect the long-term viability of wildlife populations (Andrews et al. 2008) through mortality from vehicular collisions (Fahrig et al. 1995; Aresco 2005; Glista et al. 2009), restriction of wildlife movement across the landscape (Ashley & Robinson 1996; Gibbs & Shriver 2002; Rouse et al. 2011; Brehme et al. 2013), and population isolation. The effects of roads, however, are not limited to specific environments, as high levels of mortality can be found within many urban, as well as protected areas (Ashley & Robinson 1996; Aresco 2005; Shepard et al. 2008).

Various mitigation approaches have been used to alleviate the detrimental effects of roads on wildlife (Glista et al. 2009; Rytwinski et al. 2015). Barrier fencing and underpasses are common mitigation approaches used for reptile conservation (Yanes et al. 1995; Dodd et al. 2004; Aresco 2005; Baxter-Gilbert et al. 2015). Barrier fences and crossing structures redirect reptile movement away from roadways, thus reducing direct road mortality, and promote habitat connectivity by restoring uninterrupted movement (Yanes et al. 1995). Unfortunately, inappropriately designed road mitigation structures do little to minimize the negative effects of roads and possibly even result in further negative effects on the surrounding environment and wildlife populations (Glista et al. 2009; Baxter-Gilbert et al. 2015).

Studying the ecological consequences of transportation infrastructure has been a focus for many biologists. Possible mitigation measures to reduce the negative effects of roads on the environment and individual species will be an important future direction for both planners and biologists (Mader 1984; Rosen & Lowe 1994; Gibbs & Shriver 2002). There is no universally-accepted mitigation design that is effective for every socioeconomic roadway situation or wildlife species. Therefore, wildlife biologists must work with stakeholders to ensure customized mitigation measures are designed and implemented effectively (Glista et al. 2009). Although drift fences accompanied by ecopassages are designed to accommodate the movement of various animals and provide a solution to the problem of direct road mortality, their efficacy is rarely fully evaluated (Clevenger & Waltho 2000; Lougheed & Xuereb 2013; Baxter-Gilbert et al. 2015).

Dramatic global declines are evident in many reptile and amphibian populations (Gibbons 2000; Pounds 2006; Pounds et al. 2006; Mullin & Seigel 2009; Böhm et al. 2013). Snake populations in particular have shown dramatic declines worldwide (Mullin & Seigel 2009; Reading et al. 2010). In Canada, *Sistrurus catenatus* (Eastern Massasauga rattlesnake) has experienced both major population declines and habitat fragmentation within its range, resulting in its listing as a Species At Risk in Canada (Parent & Weatherhead 2000; Parks Canada Agency 2013).

In this chapter I addresses two objectives. For my first objective I determine the effectiveness of mitigation measures in Killbear Provincial Park, specifically placed to reduce Massasauga road kill and maintain population connectivity. Mitigation efficacy was assessed using five approaches: i) pre- and post-mitigation road mortality analysis to determine changes in road mortality over time; ii) camera traps in ecopassages to determine reptile and predator presence; iii) implanting reptiles with PIT tags (Passive Integrated Transponders) and installing automated readers in ecopassages to determine reptile use; v) a willingness to utilize (WTU) experiment to assess likelihood of ecopassage use; and vi) an assessment of thermal conditions in ecopassages to determine their suitability for snakes. If the mitigation fencing is effective at preventing Massasaugas from accessing the road, I expected a significant decrease in abundance of Massasaugas on the road post-mitigation. If the ecopassages are effective at facilitating population and habitat connectivity, then I expected Massasaugas would use the crossing structures as opposed to the road to access habitat.

For the second objective I determine if further mitigation measures for reptiles should be implemented in other key areas throughout Killbear Provincial Park. This was assessed using three methods: i) a hot spot analysis to determine areas of high road mortality; ii) a carcass persistence experiment to determine road mortality survey accuracy; and iii) an analysis of seasonal road mortality.

Methods

Study area and mitigation measures

All surveys were conducted in and adjacent to Killbear Provincial Park, located in Carling Township, Parry Sound District, Ontario. Killbear is one of the busiest provincial parks in Ontario with over 250 000 visitors annually (Parent & Weatherhead 1998, 2000). Killbear is home to three species of turtles and ten species of snakes, including the Threatened Eastern Massasauga rattlesnake. After the opening of Killbear in 1960, Massasaugas were routinely killed by park staff and visitors due to safety concerns (Paleczny et al. 2005; E. Ramsey pers. comm. 2013). By the early 1970s, park staff stopped intentionally killing Massasaugas, choosing instead to relocate them long distances from campgrounds to preserve the snakes while still addressing visitor safety issues (Paleczny et al. 2005). Due to site fidelity to hibernation sites, it is likely that most of these relocated snakes died (K. Otterbein pers. comm. 2015). As of 1992 rattlesnakes were no longer relocated long distances at Killbear. By the mid-1990s, opinions about Massasaugas began to change substantially as a result of concerns about population declines and an intensive public education program. This program was intended to assist Massasauga research within the Park, which included a variety of radio telemetry and mark-recapture studies, and to encourage a change in visitor and local landowner perception of snakes (Paleczny et al. 2005). The ongoing tagging of Massasaugas by researchers and park staff has resulted in a long-term database of over 1000 captures from 1992 to the present (Parent & Weatherhead 1998, 2000; K. Otterbein pers. comm. 2013).

After conducting road monitoring through the park, park staff expressed their concern over the high levels of Massasauga road kill on park roads and opted to take mitigation measures (K. Otterbein pers. comm. 2013). Barrier fencing and ecopassages were installed in Killbear from 2007-2013 in an effort to reduce Massasauga road mortality (Figure 1.1). The initial barrier fence (400 m) was built on one side (northeast side) of the main park road (an extension of Provincial Hwy 559) in 2007. It was further extended in 2009 by 400 m and again in 2013 by 100 m. A second barrier fence (660 m) was constructed in 2011 on both sides of Day Use Road, a secondary park road that runs directly through Massasauga habitat. The third barrier fence (300 m) was constructed on both sides of Blind Bay Campground Road in 2011 and was further extended another 300 m in 2013. Fencing leads to a total of four grate-top ecopassages that were installed in 2010 and 2011. Two of these ecopassages were installed on Blind Bay Campground

Road and two were installed on Day Use Road. Light gauge metal hardware cloth (1 cm x 1 cm) was used to construct barrier fencing. Fencing was designed to be L-shaped with 75 cm extending above ground and 30 cm buried underground, including a 15 cm section bent at a right angle and projecting towards the rattlesnake habitat to prevent animals from burrowing their way underneath the fence. In wet areas, the fencing material rusted out over time and was not functional. To solve this problem, affected sections of fencing were replaced with new hardware cloth, augmented with a geotech fabric that has a 20 year life span against water and UV. Ecopassage walls and bases were constructed from concrete, while the top is an open metal grate; passage measurements are 8.5 m long with a span of 1.2 m and a height of 50-60 cm between the bottom of the grate and the backfilled substrate. In addition to barrier fencing and crossing structures, a total of four "Brake for Snakes" road signs were installed near known Massasauga road crossing locations (Figure 1.2).

Objective 1: Mitigation Efficacy

Method i: Road abundance analysis

Post mitigation monitoring of Massasauga road abundance was conducted in 2013 and 2014 by bicycle to maximize detectability of all specimens (Farmer & Brooks 2012). Roads surveyed included Park Main Road, Blind Bay Campground Road, and Day Use Road. Roads, totaling 8.4 km, were surveyed by bicycle once per day at 08:00 from mid-May to mid-June. From mid-June to 31 August, roads were surveyed by bicycle twice per day at 08:00 and again at 15:00. Bicycle surveys continued into September once per day at 08:00, and ended for the season on 10 October. Approximately 3.5 km of the Park

Main Road were surveyed nightly by car. In 2013 and 2014, night surveys were conducted once per night at 22:00 starting mid-June, and the last night survey was completed on 15 September. Surveys did not occur if there was a sustained temperature of less than 5°C during the period between surveys.

Massasaugas found during surveys were collected and location, weather, time, habitat and behaviour (if found alive) were recorded. Location was recorded in UTM using a handheld GPS receiver (Garmin, GPS 72H, Olathe, Kansas) in the NAD 83 datum. Road surface and air temperatures were recorded (Mastercraft digital temperature reader, ±1.5°C) at the time of each sighting. Live Massasaugas were weighed, measured, sexed, PIT tagged, and photographed. The snakes were weighed with a Pesola spring scale (±1 g). All snakes were photographed on a 1cm by 1 cm grid and measured using the software ImageJ (±1 cm). Sex was determined by measuring the vent to tail length and all gravid snakes were noted. Processed snakes were released less than 100 m from where they were collected. All deceased snakes were removed from the road to avoid being re-counted and they were frozen for possible future genetic work.

Massasauga data were also collected during incidental observations by researchers, park staff, wardens, campers and cottagers. Outreach programs encouraged the public to report live and dead Massasauga sightings. Only sightings that were confirmed by researchers were used in my study. Prior to the start of bicycle surveys in 2013, park staff opportunistically conducted road surveys for Massasaugas by car from May-September 2002-2012. Roads surveyed included Park Main Road (Hwy 559), Blind Bay Campground Road and Day Use Road.

Differences in abundance of Massasaugas on roads among the pre-, during- and post- mitigation study years were examined using a similar resampling technique as was used by Lougheed and Xuereb (2013). I compared the number of Massasaugas, both alive and dead, found on park roads pre- (2005-2007), during- (2008-2011) and post- (2012-2014) mitigation. Sample sizes were not consistent during the study; to alleviate this issue in part, all capture data were subsampled with replacement at random for 25 snakes, the smallest sample size (2010) in the data set, a total of 1000 times. For each year, the proportion of snakes on the road was calculated from the subsample results by dividing the total number of snakes found on the road by the sample size (25). The proportion of Massasaugas on the road pre- mitigation was compared to the proportion of Massasauga on the road during- and post-mitigation using a Kruskal-Wallis test and a *post hoc* Nemenyi Test (R Development Core Team 2014).

Method ii: Camera traps

Camera data were used to quantify the number of Massasaugas using the ecopassages. Wildlife cameras (Bushnell Trophy Max HD) were installed and operated at one entrance of each of the four ecopassages from 15 May 2013 until 6 October 2013 to capture any ecopassage use by Massasaugas and other animals (Foster & Humphrey 1995; Pagnucco et al. 2011; Baxter-Gilbert et al. 2015). Cameras were positioned such that the frame encompassed the entire entrance of the ecopassage. Cameras were programmed to take a picture when movement was detected (Pagnucco et al. 2011).

To increase capture success in 2014, a second camera was installed at each ecopassage ensuring that both openings were being monitored for snakes entering and exiting the passage. Cameras were repositioned in the 2014 field season on a large stake in front of the entrance and aimed directly at the ground of the ecopassage entrance (Pagnucco et al. 2011; Baxter-Gilbert et al. 2015). Due to the varying ranges of success with using motion sensor programming to photograph small wildlife, the cameras were programmed to take a picture at one-minute intervals, the lowest interval setting on the camera, to increase the probability of photographing snakes (Pagnucco et al. 2011; Baxter-Gilbert et al. 2015). The one-minute picture interval has been used successfully in several other ecopassage studies, but it is important to consider that detection probabilities were not perfect, as an animal may have entered the ecopassage in under a minute (Gates & Sparks 2011; Baxter-Gilbert et al. 2015). Cameras recorded images from 28 May 2014 until 6 October 2014.

Photographs taken during the 2013 and 2014 seasons were reviewed individually by a researcher to quantify the number of Massasaugas and other reptile and amphibian species using the ecopassages. Cameras also assisted in tabulating mammal species using the ecopassages to determine if predation poses a risk to Massasaugas in the ecopassages.

Method iii: Pit Tags

During the 2013 and 2014 active seasons, Massasaugas (N = 68) above 20 g found within the Park were captured and each outfitted with a subcutaneously-injected PIT-tag. Prior to this project, park staff had been PIT-tagging Massasaugas in Killbear and the surrounding area since 1993 (N = 750) for various research and monitoring projects.

Automated PIT tag readers were installed from 24 May 2013 – 11 October 2013 at one entrance of each of the four ecopassages (one AVID Industrial Model Multi-Mode

Reader and three Dorset ID LID575-Multi Custom Stationary Decoders). During the 2014 active season, from 12 May 2014 - 10 October 2014, automated PIT tag readers were once again installed at one entrance of each of the four ecopassages as well as the exit of two of the ecopassages (three AVID Industrial Model Multi-Mode Reader and three Dorset ID LID575-Multi Custom Stationary Decoders). The additional tag readers were purchased and used in the 2014 season due to increased funding.

To prevent individuals from crossing through the ecopassage without being detected, fencing was installed to ensure that the automated tag reader antennas spanned the entire entrance/exit of the ecopassage. The AVID Readers were constantly scanning and the Dorset Decoders scanned individuals after movement triggered a sensor. The readers were programmed to record the individual tag numbers, time and date of crossing.

Method iv: Willingness to Utilize (WTU)

During the 2014 field season, Massasaugas found crossing roads or actively moving through campsites and cottage lots were captured. *Thamnophis sirtalis sirtalis* (Eastern Garter snakes) were also opportunistically captured and used in this experiment. These two species of snakes were tested because different species can exhibit varying levels of willingness to cross open areas, roads, and presumably ecopassages as well, due to instinctive behaviours based on life history traits (Andrews & Gibbons 2005). Both Massasaugas and Garter snakes demonstrate road avoidance behaviour, but species that rely on crypsis, such as the Massasauga, are expected to move more slowly in threatening situations (Parent & Weatherhead 2000, Andrews & Gibbons 2005, Eads 2013).

To minimize stress and handling, snakes were not processed (measuring, PIT tagging, etc.) until after use in the WTU experiment. As per similar studies, prior to the experiment, snakes were placed into an open top - open bottom garbage bin and given a five-minute acclimation period directly in front of the ecopassage (Andrews & Gibbons 2005; Woltz et al 2008; Hamer et al. 2014; Baxter-Gilbert et al. 2015). After the acclimation period, the box was opened using a rope and pulley system activated by a researcher from a distance, where they would not be detected by the snake and therefore were presumably not influencing the snake's behaviour (Andrews & Gibbons 2005; Baxter-Gilbert et al. 2015). The area surrounding the garbage bin and entrance was fenced to both prevent the snake from escaping and to provide motivation to use the ecopassage. A large section at the exit of the ecopassage was also enclosed to ensure that the snake did not escape and could be recaptured once the WTU test was completed.

Once the garbage bin was lifted, the snake's movements and behaviours were observed. The snake's response to the ecopassage was based on a similar scoring system to that of Lesbarrères et al. (2004) and Baxter-Gilbert et al. (2015). The response to the ecopassage was ranked on a scale of 0-5 of crossing success: 0) not willing to use; 1) made no choice, stayed near entrance; 2) experimented with use, entered first quarter of ecopassage; 3) crossed half of ecopassage, 4) crossed three quarters of ecopassage; and 5) completely willing to use, crossed full length of ecopassage. After 45 minutes the snake was captured and given a score based on its last position (Baxter-Gilbert et al. 2015).

This test was conducted *in-situ* on an active campground road as it was not possible to close the road during experiments. Therefore there was no standardization of traffic over the ecopassage; although this introduces and uncontrolled variable, it allowed me to observe realistic behavioural responses to various traffic volumes. Temperature can be considered a second uncontrollable variable as temperature is known to affect snake defensive behaviour (Brodie & Russell 1999). Because experiments were conducted at various times through the summer, temperature may have been a factor in the outcome of the experiments. Although this park road has minimal pedestrian traffic, signage was placed on both sides of the road to ensure minimal disruption to researchers from the public. After the WTU test, the snake was processed and tag numbers were recorded to avoid using the same test subject again. Following the processing, all snakes were then returned to their original site of capture.

A Chi-squared test was used to compare the number of Massasaugas to the number of Garter snakes that: a) did not use the ecopassage (0-1), b) entered the ecopassage but did not fully cross in the allotted time (2-4), and c) that completely crossed the full length of ecopassage (5).

Tunnel usage and tunnel efficiency rates for both Massasaugas and Garter snakes were calculated. Tunnel usage is defined as the proportion of individuals that entered the ecopassage, and either completed or did not successfully complete crossing (Hamer et al. 2014). Tunnel efficiency is defined as the proportion of individuals that traveled through the entirety of the ecopassage and exited at the opposite end (Hamer et al. 2014).

Method v: Thermal Suitability of Ecopassages

During the 2014 active season, all ecopassages were outfitted with iButton temperature data loggers (iButton DS1921G Maxim Integrated, ±1°C) to investigate the ambient thermal patterns within the ecopassages, as well as the surrounding environment.

Microclimates and unsuitable thermal environments have been suggested as possible reasons for the reluctance of herpetofauna to use culverts (Langton 1989; Reading et al. 2010). The open grate design of Killbear's ecopassages suggests that differences between microclimate inside and outside will be minimal. Data loggers were placed in three locations to capture any potential temperature differences surrounding and within the ecopassages: 10 m directly behind the entrance on the forest floor, ground level at the ecopassage entrance, and on the road surface beside the ecopassage. Four data loggers were placed equidistantly (approximately 1 m apart) inside the ecopassage.

The temperature data were summarized monthly (June-September) and divided into three diel time periods per month. The time periods were defined as morning (sunrise to 11:59), afternoon (12:00 to sunset) and night (sunset to sunrise). ANOVAs with a *post hoc* Tukey test were used to compare temperatures within each ecopassage (mean of four data loggers placed equidistantly inside the ecopassage) to their surrounding environment (road, forest, entrance) for each month.

The mean temperature of each ecopassage was compared to three known Massasauga thermal tolerance ranges, to determine if and when ecopassages satisfy Massasauga thermal requirements. The three known tolerances identified were: the activity range, the preferred range, and the performance range. The activity range is the temperature range that can be tolerated when above ground (based on the lowest and highest body temperatures observed in the field), and for Massasaugas was determined to be 1.1°C to 44.0°C (Harvey & Weatherhead 2010, 2011). The preferred temperature range is the temperature range within which a snake would ideally maintain their body temperatures in the absence of factors that would influence temperature selection in the wild (Harvey & Weatherhead 2010, 2011). Biological functions are optimized in the preferred temperature range, which for Massasaugas is 30.0°C to 33.6°C (Harvey & Weatherhead 2011). The performance range is defined as the range of temperatures within which most functions are completed reasonably well; the lower limit of this range was set at the temperature below which Massasaugas had less than 50% probability of moving between locations (19.9°C) (Harvey & Weatherhead 2010, 2011).

Massasauga crossing times and temperatures in 2014 were summarized to determine if there was a specific time or temperature range that coincided with ecopassage crossings. The mean ecopassage temperature at each time of crossing was also compared to the species' known activity range, preferred range, and performance range (Harvey & Weatherhead 2010, 2011).

Objective 2: Future Mitigation

Method i: Hot Spot Analysis

To fully assess Killbear's reptile road mortality mitigation needs, road surveys for snakes and turtles were completed on all main park roads and boundary roads in 2013 and 2014. Roads surveyed included Main Road (Hwy 559), Blind Bay Campground Road, Day Use Road, Blind Bay Cottage Road, Pengally Bay Road, and Linda Lane (25 km in total). Road surveys were conducted at the same time and in the same manner as the Massasauga road surveys, as described for Objective 1.

Determining the locations of road kill hot spots is essential to direct effective mitigation on roadways (Clevenger et al. 2003). The annual numbers of alive and dead on road reptiles on park (non-fenced roads) and cottage roads were tabulated for the 2013 and 2014 active seasons (Shepard et al 2008). Using SIRIEMA software, I identified reptile road mortality hot spots and determined specific potential sites that require mitigation (Coelho et al. 2012; Teixeira et al. 2013). Single species-based analysis were not completed as I had too few data for some species. To minimize issues regarding the conservation importance of individual species, a ranking system was used to assign greater importance to endangered, threatened and special concern species (Teixeira et al. 2013). Hot spot data were mapped onto the most current satellite imagery of Killbear using ESRI ARC Map (ESRI 2014).

Average hourly traffic rate was estimated through various count surveys conducted over the course of the active season in 2014. Count surveys involved a researcher counting vehicles entering and exiting Park Main Road, Blind Bay Cottage Road and Pengally Bay Road. Count surveys were conducted for 30 minutes. Surveys occurred on 57 occasions for a total of 28.5 hours by researchers, during working hours of both weekdays and weekends (8:30-4:00) of June, July and August. Traffic rate was then averaged and calculated as number of cars per hour, per day.

Method ii: Carcass Persistence on Roads

As carcasses can be scavenged or decompose before being observed by researchers, the duration of time that a carcass persists on a roadway is an important factor in road mortality estimates (Slater 2002; Santos et al. 2011; Hubbard & Chalfoun 2011; Guinard et al. 2014). During the 2014 active season, intact Garter snake carcasses were opportunistically collected and positioned on the roadway to observe carcass persistence. Carcasses were deployed in five suspected hot spot locations on park roads throughout the course of the active season. When a carcass was placed at a designated site on the road, the body condition and positioning on the road were recorded. Wildlife cameras, set to detect movement, were deployed beside the carcass to observe length of persistence and scavenger species. Researchers also frequently rechecked carcass absence/presence.

A non-parametric product-limit survival analysis (Kaplan-Meier estimator) was used to calculate maximum persistence time, average persistence time in hours, and an estimate of persistence probability between surveys (Santos et al. 2011; Riley & Litzgus 2014; R Development Core Team 2014).

Method iii: Seasonal Variation in Road Mortality

To analyze seasonal variation in reptile mortality, monthly frequencies of total road mortality for the 2013 and 2014 field season were tabulated, controlling for effort through daily surveys. Pearson correlation coefficients were calculated to test if monthly frequencies of dead on road reptiles correlated with monthly park visitation (Shepard et al. 2008). Monthly park visitation was compiled by park administration using vehicle permits sold (Ontario Parks 2013, 2014).

Results

Objective 1: Mitigation efficacy

Method i: Road abundance analysis

The proportion of Massasaugas on the road (alive and dead) out of the total caught per year, was lower during the construction of mitigation (2008-2011) compared to pre-mitigation levels (2005-2007). Post-mitigation, during the focused research years (i.e., during my thesis fieldwork; 2012-2014), there was a further decline in the proportion of Massasaugas on the road below the pre-mitigation and construction of mitigation levels (Figure 1.3).

The Kruskal-Wallis test indicated there was a significant effect of mitigation on road mortality ($p=0.018$, $df=2$). The Nemenyi *post-hoc* test determined that there is a significant difference between the pre- and the post-mitigation major research years ($p=0.013$). These analyses suggest that mitigation efforts have resulted in a significant reduction in abundance of Massasaugas on roads.

Methods ii & iii: Camera traps & PIT tags

In 2013, a total of nine non-reptile species were detected using the ecopassages (Figure 1.4). The motion-triggered cameras did not capture any reptile or amphibian species. The highest recorded use by mammals was in Ecopassage 3 followed by Ecopassage 2, Ecopassage 4 and Ecopassage 1.

In 2014, a variety of herpetofauna species, including frogs, salamanders, snakes and a turtle, was detected using the ecopassages (Figure 1.5). The highest recorded use by reptiles/amphibians was in Ecopassage 4 followed by Ecopassage 1, Ecopassage 3 and Ecopassage 2. The majority of frog and salamander photographs were not identifiable to species level, due to low-resolution night pictures. Species that were identified included *Lithobates clamitans* (Green frog), *Anaxyrus americanus* (American Toad), *Plethodon cinereus* (Eastern Red-backed salamander), *Ambystoma laterale* (Blue-spotted salamander) and *Ambystoma maculatum* (Spotted salamander). Snake species observed included *Sistrurus catenatus* (Massasauga Rattlesnake), *Pantherophis gloydi* (Eastern Foxsnake), *Nerodia sipedon sipedon* (Northern water snake), *Thamnophis sirtalis sirtalis* (Eastern Garter snake) and *Storeria* sp. The only turtle observed was a juvenile *Chelydra serpentina* (Snapping turtle).

In 2013, a total of five PIT-tagged Massasaugas were recorded in ecopassages by the automated PIT tag readers. Four of these were recorded in Ecopassage 4 and one was recorded in Ecopassage 2. The wildlife cameras did not capture these crossings.

A total of nine Massasaugas were recorded in ecopassages in 2014. Eight of these were recorded in Ecopassage 4 and one was in Ecopassage 3. Of these nine records, three were simultaneously recorded on both the tag readers and cameras. Four of these 10 readings were only recorded on the camera and not the tag reader. Two of the nine readings were recorded by the tag reader and not the camera. An additional Massasauga (tenth) was captured by a camera at Ecopassage 4, but it could not be determined if the snake actually entered the ecopassage.

Two Foxsnakes were recorded in ecopassages during the 2014 study year. One observation was in Ecopassage 4 and one was in Ecopassage 3. One was recorded by the camera and not by the tag reader and the second was recorded by the tag reader and not the camera. A juvenile Snapping turtle was recorded by cameras crossing Ecopassage 1.

Method iv: Willingness to Utilize (WTU)

Most Massasaugas tested (*N*=18/19) were willing to enter the ecopassage. Just over 1/3 of individuals crossed completely (*N*=7/19; 36.8%), while the majority did not fully cross in the allotted time (*N*=11/19; 57.9%). One individual was not willing to use the ecopassage to any extent (5.3%).

Garter snakes showed similar crossing tendencies to Massasaugas. The majority of Garter snakes were willing to use the ecopassage (*N*=10/16; 62.5%), but did not finish crossing, while 31.3% (*N*=5/16) completely crossed the ecopassage during the allotted time and only 6.3% (*N*=1/16) refused to enter.

There was no significant difference in crossing success between Massasaugas and Garter snakes ($X^2 = 0.13$, p=0.94). Massasaugas had a tunnel usage rate of 0.95 and Garter snakes had a rate of 0.94. Tunnel efficiency rates were lower than usage rates, with Massasaugas at 0.37 and Garter snakes at 0.31.

Method v: Thermal Suitability of Ecopassages (Table 1.1)

Significant temperature differences between the road, ecopassage, and entrance were primarily evident in June during all diel time periods. July and August exhibited this same trend but primarily in the afternoon and night, while September temperature differences primarily occurred in the afternoon (Appendices 1.1, 1.2, 1.3, 1.4).

During the entirety of the study period, temperatures inside the ecopassages did not extend outside the activity range of Massasaugas (1.1°C to 44°C; Harvey & Weatherhead 2010, 2011). The observed mean temperature range in Ecopassage 1 was 13.1°C to 20.9°C; Ecopassage 2 was 12.61°C to 24.62°C; Ecopassage 3 was 13.2°C to 21.5°C and Ecopassage 4 was 12.81°C to 21.02°C. Mean temperatures in each ecopassage did not reach the lower limit of the Massasauga performance range of 30°C (Harvey & Weatherhead 2010, 2011) during any diel period. The lower limit of the preferred range of Massasaugas (19.9°C; Harvey & Weatherhead 2010, 2011) was reached during the afternoon time periods in June, July and August. The mean temperature in the morning and night periods did not meet the performance needs of Massasaugas. During the entirety of September, the mean temperature during all time periods did not reach the performance minimum of Massasaugas.

Crossing Times (Table 1.2)

All nine ecopassage crossings in 2014 occurred during the afternoon, between 12:47 and 17:32. Eight out of 9 crossings occurred within a 3 hour time span (14:46-17:32). Of the 9 crossings, 5 occurred at times when the mean ecopassage temperature exceeded the lower limit of Massasauga performance temperature (19.9 °C). The remaining 4 crossings occurred at times below the performance range but during seasonally cooler time periods (early June and September).

Objective 2: Further Mitigation

Method i: Hot Spot Analysis

The abundances of all reptile species surveyed on roads (Table 1.3) were used to determine the locations of hot spots on the Park Main road, Pengally Road, Linda Lane and Blind Bay Cottage Road. Park Main Road has three identifiable hot spots, 100 m, 800 m and 50 m in length (Figure 1.6A). This road has an estimated average daily traffic of 158.3 vehicles/hour during working hours (8:30-4:00) of the high park visitation season. Pengally Road has one identifiable hot spot that is 200.25 m in length (Figure 1.6B) and an estimated daily traffic of 31.5 vehicles/hour. Blind Bay Cottage Road has one identifiable hot spot that is 400.25 m in length (Figure 1.6C) and has an estimated daily traffic of 22.3 vehicles/hour.

Method ii: Carcass Persistence

The persistence time of 20 road-kill Garter snakes was analyzed and plotted as carcass persistence overtime (Figure 1.7). After the first day (24 hours), the probability of a carcass persisting on the road was 50%. Persistence probability drops to 35% by day 2 (48 hours). The maximum persistence time of a carcass was 240 hours. The time that lapsed between road surveys in my study was 6.5 hours (morning-afternoon) and 16.5 hours (afternoon-morning). Therefore, the maximum time a carcass could potentially be left dead on the road was 16.5 hours, corresponding to a 90% probability of persistence.

Method iii: Seasonal Variation

Visitation at Killbear was lowest during the spring and fall (June and September) and highest in July and August. Figure 1.8 demonstrates that road mortality was highest during periods when visitation was highest (July, August). Mean monthly reptile mortality was positively correlated with mean monthly visitation (Pearson's correlation coefficient, $r=0.81$, $t=3.3$, $df=6$, $P=0.016$).

Discussion

Effectiveness of fencing

The fencing installed in Killbear was effective at minimizing access to roads by Massasaugas, as the proportion of total Massasaugas on the road decreased significantly over time as mitigation measures were constructed (N_{2005}=0.24, N_{2014}=0.017). The number of Massasaugas on the road fluctuated post initial mitigation; this may be attributed to the extent of fencing installed and the timing of construction. After the initial installation of fencing in late 2007, abundance on roads decreased in 2008. After the first year of mitigation, road abundance increased in 2009 and again in 2010 although it was lower than pre-mitigation levels (2005-2007). The increase in road abundance in 2009/2010 may have been because the fencing was not of adequate length to reduce access to the road. During the early stages of mitigation, the minimal fencing may have allowed Massasaugas to cross the road in new locations. The initial fencing was only installed on one park road; therefore the increased abundance on roads seen in 2009/2010 may have also been indicative of other Massasauga crossing locations in the park.

In 2011, fencing and crossing structures were installed on two secondary park roads that had known Massasauga road mortality. After the majority of mitigation was completed in 2011, there was a decrease in road abundance by 8.8% (2012). Minor additions of fencing in 2013 resulted in a small decrease in road abundance by 0.22%. Since 2012 there have been only minor fluctuations in road abundance, indicating that fencing is of adequate length to encompass most Massasauga habitat and dispersal routes that bisect roads.

Although a reduction of road mortality was observed post installation of fencing, there are other uncontrollable factors that may have affected changes in snake abundance or activity. A change in Massasauga behaviour in response to the fencing should be considered. In addition, previous road mortality may have resulted in fewer snakes being available to cross the road, resulting in an apparent reduction of mortality over time.

The success of the Killbear fencing comes with a significant caveat: it required continual labour-intensive monitoring and repair. Thus, the Killbear fencing should not be considered an effective solution for all road mitigation scenarios. The fencing was specifically built for Massasaugas and does not prevent smaller bodied snakes from gaining access to the road (i.e., small snakes, including juvenile Massasaugas can fit through the chicken wire) and therefore should not be used for a multi-species road project. The success of Killbear's fencing can be attributed to the significant labour and financial commitments of park staff and researchers. The fence was surveyed on average 14 times/week in 2013 and 5 times/week in 2014. The continuous monitoring of the fence resulted in the immediate discovery and repair of holes and tears. Prior to yearly spring emergence and dispersal of Massasaugas, extensive time was spent repairing fencing from major winter damage. Thus, this fencing style should only be considered in a situation where constant upkeep and monitoring are practical, and where maintenance labour and funds can be secured well into the future. Killbear fencing was successful in this circumstance and may work in scenarios with similar resources; however, many other studies have documented high success rates with varying taxa and alternative styles of fencing (Foster & Humprey 1995; Dodd et al 2004; Aresco 2005).

Although Killbear's fencing has resulted in a significant decrease in road abundance, four Massasaugas gained access to the road in 2013 and five in 2014. The overall condition and age of the fencing may be reducing its effectiveness. Naturally-wet areas of the landscape have resulted in the rotting and sinking of portions of the fencing. Black bears (*Ursus americanus*) and campers have repeatedly trampled areas of fencing resulting in weak, and occasionally open, sections. Despite best intentions to properly maintain the fencing and rectify these issues, damage can still go unnoticed for periods long enough to allow snakes to access the road.

A solution for many of the problems I observed with the fencing design in Killbear would be to replace fencing over time with a more durable long-lasting fence with minor readjustments in fencing locations to avoid wet areas. Smaller-bodied snakes and amphibians should be considered when choosing fencing material. The initial cost may be high for the installation of a stronger and more permanent fence, but in the long term, this mitigation option would be more cost effective when compared to the annual maintenance that is currently occurring in the park (Aresco 2005).

Effectiveness of Ecopassages

The Killbear ecopassages appear to be promoting Massasauga population and habitat connectivity. During my two-year study, a total of 14 Massasaugas were recorded using the ecopassages. There were only three occasions (N=3/14) when both the cameras and tag readers simultaneously recorded a Massasauga crossing, indicating that a single monitoring method (camera or tag reader) would not be adequate. Although automated tag readers and cameras were regularly examined and maintained, they occasionally experienced lapses in monitoring due to battery failure, memory card failure, overheating and vandalism. Overall, the survey methods used to monitor the ecopassages obviously yielded relevant data; however, the true number of individuals utilizing the crossing structures may be higher than what was actually observed due to the reasons stated above.

Ecopassages are commonly thought to be safer travel routes for wildlife compared to roads (Little et al. 2002). However, many studies raise concern that the effectiveness of ecopassages may be reduced due to the threat of predation to individuals using the crossing structures (Little et al. 2002, Ford & Clevenger 2010, Pagnucco et al. 2011). Although there are few studies that have directly tested for predation in ecopassages (but see Baxter-Gilbert et al. 2015), it is generally understood that concerns of predation are built on anecdotal evidence and observations of infrequent and incidental opportunism by predators rather than concrete data on the frequent predation (Little et al. 2002; Pagnucco et al. 2011).

Few ecopassage efficacy studies have reported predation events during monitoring (Little et al. 2002; Ford & Clevenger 2010). During my study I observed no predation events or indirect evidence of predation within or near the ecopassage. In both study years, individual Massasaugas were recorded crossing in an ecopassage multiple times, indicating crossing success without predation. In Ecopassage 4, known Massasauga predators, Raccoons (*Procyon lotor*) and Fishers (*Martes pennanti*), were recorded using the crossing structure on 42% of the monitoring days in 2013, while Massasaugas were recorded using this passage only 2.7% of the monitoring days. Considering the frequency of Massasauga crossings, it would unfeasible for a predator to develop hunting strategies for Massasaugas involving the ecopassages. As opposed to using the ecopassages as a feeding location, the high frequency of mammal traffic more likely indicates that individuals have simply incorporated them into their daily movement and home territories (Little et al. 2002). Mammalian usage of Killbear's ecopassages may be explained by the individual species' preferences for the surrounding habitat type and larger scale predator-prey interactions occurring in the habitats bisected by roads (Little et al. 2002).

Although a specific hunting tactic for Massasaugas is unlikely, predation should still be considered a possibility. A wide variety of mammals and reptiles/amphibians were using the ecopassages, creating an area of concentrated prey. Food abundance, such as a concentration of resources, is known to affect the movement and distribution of Raccoons (Prange et al. 2004). It is plausible that a predator such as a Raccoon could target the ecopassages due to high usage by other prey species, resulting in the opportunistic capture of Massasaugas. The issue of prey traps should be considered in the future, should additional observations indicate that prey are being exploited by predators in and around the ecopassages (Little et al. 2002).

Results from the WTU test demonstrate that Massasaugas (N=95%) and Garter snakes (N=94%) were willing to use the ecopassage, supporting patterns seen with the wildlife cameras and automated tag readers. Usage rates in my experiment differed from the results obtained in other studies and species: Painted turtles; N=0.09, (Baxter-Gilbert et al. 2015), Green frogs; N=0.68, Leopard frogs; N=0.77, (Woltz et al. 2008) and Spotted salamanders; N=0.76 (Jackson & Tyning 1989). In my study, only 12 out of 33 snakes (36%) that entered the ecopassage exited the opposite side in the allotted time. Due to the difficulty of providing motivation for a snake to cross an ecopassage in an experimental situation, it is difficult to discern why a large portion of snakes did not finish crossing the ecopassage. Perhaps given more time, the snakes would have completed crossing, as Massasaugas and other viperid snakes are known to move slowly across roads (Seigel & Pilgrim 2002, Andrews & Gibbons, 2005). Staged experiments may also result in hesitancy from the snake to complete crossing (Andrews & Gibbons, 2005). As snakes were willing to enter the ecopassage, it is plausible that in a natural setting, given biologically relevant motivation, more snakes would complete the crossing.

Although different snake species might be expected to exhibit varying levels of willingness to move through habitats depending on their life history attributes (Andrews & Gibbons 2005), I did not observe this. Both Massasaugas and Garter snakes showed similar willingness to use the ecopassages. This is further supported by wildlife camera observations of multiple other snake species using the ecopassages as well as multiple amphibian species and a turtle. The willingness of various reptile and amphibian species to use the crossing structures suggests that the ecopassage design is effective and presents a non-threatening transportation corridor to reptiles and amphibians.

Roads are often exposed to direct sunlight and are constructed of artificial materials that absorb heat and hold it longer than many natural surfaces (Shine et al. 2004). Ectotherms such as snakes require various levels of heat for thermoregulation and therefore are often drawn to roads (Ashley & Robinson 1996; Enge & Wood 2002; Shine et al. 2004). Roads are not only used by snakes for thermoregulation, but also to access resources on alternate sides of the road (e.g. gestation sites, hibernacula, prey). Barrier fencing restricts access to the road, limiting snakes from using it as a basking site and crossing to adjacent resources. An ecopassage provides the opportunity to regain access to isolated resources (Foster & Humphrey 1995; Alexander & Waters 2000; Clevenger & Waltho 2000). There are several factors that influence ecopassage use, such as light and temperature inside a crossing structure (Langton 1989; Yanes et al 1995). Historically high road mortality of Massasaugas in Killbear indicated that snakes were using the road to gain access to resources. To replicate the form and function of the road, Killbear ecopassages were designed with open grates to allow natural light, ventilation and temperature moderation inside the ecopassage (K. Otterbein pers. comm. 2013).

Although the open-top design of the Killbear ecopassages suggests a more appropriate thermal environment than a traditional box culvert, the ecopassages still present an environment that is thermally different than the surrounding area (forest, entrance, road). The temperature variation between the ecopassages, forest and entrance hopefully mimics natural temperature differences throughout a habitat caused by the sun's position and overhanging vegetation at each site and is not a severe enough gradient to deter crossing. While a temperature gradient exists between the ecopassages and surrounding environment, the ecopassages were still thermally suitable to Massasaugas. The ecopassages would be inhospitable if the temperatures inside did not coincide with the activity temperature range needed for above ground movement in snakes. Since temperatures inside ecopassages were within the activity range during the entire study, it was biologically plausible that snakes could use the ecopassages, as they did not present a potentially dangerous and unpassable thermal environment. However, mean temperatures inside the ecopassages did not reach the preferred thermal temperature range of Massasaugas during any of time period. This does not pose a concern, as the preferred temperature range is needed only for optimal biological functions and Massasaugas are capable of functioning at temperatures far outside their optimal range (Huey & Kingsolver 1993; Harvey & Weatherhead 2010, 2011). Therefore this preferred temperature range is not required within the ecopassages to facilitate use. It is most ecologically relevant to base the assessment of ecopassage thermal environments on temperatures at which reptiles are capable of performing reasonably well as opposed to the optimal temperature. This is especially true of species at their northern range limit that need to perform over a broad range of temperatures, as is the case in Killbear (Huey & Kingsolver 1993; Harvey & Weatherhead 2010, 2011). Therefore the most appropriate measure of thermal suitability in the ecopassages is the performance range of Massasaugas, which directly relates to probability of movement (Harvey & Weatherhead 2010, 2011).

Massasaugas are typically crepuscular during summer months (Shepard et al. 2008); I thus expected ecopassage travel to occur in the morning and/or evening, but found that all recorded crossings occurred in the afternoon. I found that ecopassages had a thermal environment falling inside the performance temperature range only in the afternoon, when all Massasauga crossings occurred, rather than in the morning or evening. All Massasauga crossings occurred within a short time period in the afternoon, implying that they may all be coincident with a period when ecopassages are closest to an ideal thermal environment. A study of Massasauga body temperatures in the Bruce Peninsula determined that body temperatures are typically in the performance temperature range in June, July and August from 12:00 to 20:00 and in September from 1:00 and to 18:00 (Harvey & Weatherhead 2010, 2011). All the Massasauga crossings in Killbear occurred within these time frames, implying that crossings may be more likely to occur when Massasauga body temperatures are within the performance range.

Although temperatures in the morning and night periods were not within the species' performance temperature range, movement through the ecopassages is still plausible in these time periods. Below the lower limit of the performance temperature range (19.9°C), there is still a 50% probability of movement (Harvey & Weatherhead 2010). Therefore movement still occurs, but the probability of movement steadily and quickly decreases as the temperature drops. During the fall, temperatures within ecopassages are not sustained in the performance range as was the case in the summer, but body temperatures and thermoregulatory efforts of Massasaugas decrease, as most aspects of their life cycle are complete, making the maintenance of higher temperatures less important (Harvey & Weatherhead 2010, 2011). These considerations together suggest that ecopassages may still present a suitable thermal environment during the cooler months. The Killbear ecopassage design may be effective as a transportation corridor for other snake species as well, because thermal requirements of Massasaugas tend to be higher than those of other species (Harvey & Weatherhead 2011). Ecopassages thus probably present a suitable thermal environment to other snakes.

Future Mitigation

After two years of standardized surveys at Killbear, five hot spots on the survey route, ranging from 50-800 m in length, were identified. These reptile hot spot locations should be further evaluated as potential future mitigation sites. Identifying road kill hot spots is essential to implementing successful mitigation on existing roadways (Teixeira et al. 2013), and the efficacy of mitigation measures depends on their placement in the landscape (Glista et al. 2009). After identifying locations of high road mortality, options for mitigation will be well informed and can be planned and implemented effectively (Clevenger et al. 2003; Teixeira et al. 2013).

I recommend that Killbear consider mitigation fencing covering the hot spot on the Park Main Road. The type of mitigation fencing installed should be designed to prevent small-bodied snakes and turtles from accessing the road. An ecopassage should replace the current structural culvert on the main park road beside the Blind Bay parking lot. Further research should be conducted into the type of ecopassage necessary for this location to ensure it facilitates snake, turtle and amphibian movement while meeting the hydrological needs of the wetland and the structural requirements of the road.

I also recommend that Killbear informs Carling Township of the potential road mortality issues on the two cottage (Township operated) roads surrounding the park (Blind Bay and Pengally) to ensure that the Township is aware of these issues and a working relationship can be formed. Mitigation structures might be difficult to install on these roads due to property access issues. I recommend continued monitoring of hot spot locations. Before mitigation attempts are warranted, I suggest a public awareness campaign to inform cottagers of the current situation. Given that most cottagers were very cooperative during my project, I believe the installation of signage in hot spot locations would increase driver awareness of snakes on the road and could potentially prove effective.

Survey Accuracy

To increase detection rates during road surveys, I chose bicycling over driving, as detecting carcasses by bike is known to be more effective than by car (Kostecke et al. 2001; Santos et al. 2011; Farmer & Brooks 2012). However, despite best efforts to increase detectability while surveying, no method completely compensates for carcass removal by scavengers prior to detection (Hubbard & Chalfoun 2012). Many forms of wildlife actively scavenge along roads and rapidly remove carcasses, resulting in an underestimation of road mortality (Hubbard & Chalfoun 2012). Poor detection rates will impact the accuracy of road morality estimates and negatively affect management and conservation decisions (Meunier et al. 2000; Kostecke et al. 2001; Slater 2002; Hubbard & Chalfoun 2012). During my study, I found 50% carcass removal within the first 24 hours. Other studies have shown a variety of differing persistence rates, including rapid removal of carcasses within 8-12 hours, 97% removal within 36 hours, and 75% removal within 60 hours (Hubbard & Chalfoun 2012). Thus, standardizing carcass removal rates across studies is difficult as carcass persistence depends on scavenger abundance, which is site specific (Ponce et al. 2010). Although scavengers can negatively affect road mortality reports, the severity depends on timing of the surveys (Hubbard & Chalfoun 2012). As the maximum time between surveys in my study was 16.5 hours, corresponding to a 90% carcass persistence estimate, at most, only 10% of carcasses were potentially not detected, resulting in a high detectability rate. Thus, because I surveyed by bicycle and surveyed frequently, my estimates of road mortality should be accurate.

Seasonal Variation

Greater visitation during summer months (July and August) coincided with higher incidents of road mortality on park roads, confirming the importance of conducting more frequent road surveys during summer months. Several studies also found that increased traffic coincides with high road mortality of most taxa, including Massasaugas (Fahrig et al. 1995; Seigel 1986; Hels & Buchwald 2001; Szerlag & McRobert 2006). Knowledge of high mortality seasons can allow stakeholders to focus management actions during these peak periods of road mortality and implement solutions such as lower seasonal speed limits or temporary speed bumps (Shepard et al. 2008).

Conclusion

My research into the effectiveness of Killbear Provincial Park's road mitigation structures determined that Massasauga road mortality has decreased over time and that the ecopassages are indeed being used by Massasaugas and a variety of other reptiles and amphibians. Further, results of the behavioural study bolster this observation as they indicated that snakes are willing to use the Killbear ecopassages. The ecopassages had a suitable thermal environment to facilitate ecopassage travel by Massasaugas. The caveat of these positive findings is the required maintenance schedule for current fencing; over my study the efficacy of the fencing depended heavily on intense annual maintenance. In addition, smaller-bodied reptiles were able to pass through the current fencing due to the large mesh size, a consideration for future fence design.

As human population increases and continues to create demands for more road infrastructure, ecologists are confronted with the challenge of balancing increases in road construction and traffic volumes with species' protection and prevention of reptile declines on micro- and macro-geographic scales (Gibbs & Shriver 2002). Construction of wildlife exclusion and connectivity structures is becoming increasingly popular to reduce threats of roads to reptiles (Jaeger & Fahrig 2004; Dodd et al. 2004; Aresco 2005; Baxter-Gilbert et al. 2015). However, to effectively protect reptiles from these threats, additional research needs to be directed towards quantitatively assessing the effectiveness of road mortality mitigation (Glista et al. 2009; Lesbarrères & Fahrig 2012; Baxter-Gilbert et al. 2015).

It is crucial to consider that the effectiveness of mitigation measures depends on the level of commitment and dedication of all stakeholders. Without committed time and resources from all parties, mitigation is not feasible. Post-mitigation monitoring is as important as implementation of mitigation measures as it strengthens the support of mitigation methods, which can be used in other key areas to further reduce road mortality and ensure habitat connectivity across landscape.

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Table 1.1: Mean environmental temperatures (℃) per month and diel time period for Killbear Park Ecopassages.

Month	**Ecopassage**	**Inside Ecopassage**			**Entrance**			**Forest**			**Road**		
		Morning	After-noon	Night	Morning	After-noon	Night	Morning	After-noon	Night	Morning	After-noon	Night
June	1	15.33	20.94	14.94	15.51	21.25	14.37	15.5	19.19	14.1	16.13	23.1	15.53
	2	17.33	20.59	16.33	16.44	29.26	14.94	16.12	18.71	13.96	18.02	29.02	16.59
	3	15.89	21.53	14.7	20.12	27.89	15.5	16.87	20.12	14.52	17.54	27.05	16.17
	4	16.61	21.02	14.86	16.21	22.15	14.49	18.12	21.96	14.44	19.13	25.62	16.76
July	1	16.36	20.33	15.92	15.96	20.27	15.27	16.23	19	15.25	16.17	21.3	15.96
	2	17.35	24.62	15.49	16.57	23.43	15.33	16.21	17.73	15.22	17.92	23.96	16.49
	3	16.75	19.9	16.27	18.06	22.45	16.44	16.96	18.58	15.97	17.67	23.85	17.08
	4	17.12	20.01	16.1	17.06	20.96	16.26	18.07	21.16	16.11	18.6	23.21	17.14
August	1	16.56	20.45	16.44	16.41	21.62	15.85	16.8	19.85	15.71	16.28	21.78	16.15
	2	16.86	20.8	16.46	16.5	23.47	15.41	16.67	19.97	15.97	17.07	24.01	16.57
	3	16.52	21.12	16.07	16.95	22.37	16.24	17	20.13	15.8	17.89	25.06	17.31
	4	16.94	20.17	15.97	17.43	21.94	16.25	16.5	22.67	16.15	17.67	24.71	17.13
September	1	13.12	16.51	13.14	13.28	17.47	13.26	13.68	16.31	13.09	13.12	18.07	13.11
	2	12.61	15.08	12.66	12.61	17.08	11.98	12.89	15.56	12.85	12.88	17.15	12.87
	3	13.22	16.35	13.21	13.64	18.89	12.46	14.05	17.59	12.95	13.94	19.3	13.67
	4	13.15	16.62	12.81	13.82	17.95	13.18	13.93	18.96	13.33	13.8	19.92	13.42

Table 1.2: Time and mean temperature inside ecopassage at time of Massasauga (*Sistrurus catenatus*) ecopassage crossings in 2014 in Killbear Provincial Park.

Crossing Date	**Time**	**Time Period**	**Mean Temp** (°C)
June 3	16:00	Afternoon	16.83
June 26	17:32	Afternoon	21.75
June 28	16:25	Afternoon	26.33
August 11	17:14	Afternoon	25.29
August 24	15:22	Afternoon	26.44
September 6	14:46	Afternoon	19
September 7	12:47	Afternoon	21.42
September 16	16:15	Afternoon	13.83
September 17	15:00	Afternoon	15.75

Table 1.3: Species and number of reptiles, alive and dead, observed on the surveyed roads in Killbear Provincial Park during 2013 and 2014 (May-October).

Species	**Dead on Road**		**Alive on Road**	
	2013	**2014**	**2013**	**2014**
Thamnophis sirtalis	127	101	17	10
Diadophis punctatus	17	17	0	1
Storeria occipitomaculata	13	17	0	1
Sistrurus catenatus	8	9	7	5
Nerodia sipedon sipedon	10	14	1	4
Storeria dekayi	8	8	1	1
Chrysemys picta	3	1	2	8
Pantherophis gloydi	5	2	4	3
Lampropeltis triangulum	3	1	0	0
Opheodrys vernalis	1	3	0	0
Chelydra serpentina	0	0	2	7
Emydoidea blandingii	0	0	0	4

Killbear Mitigation

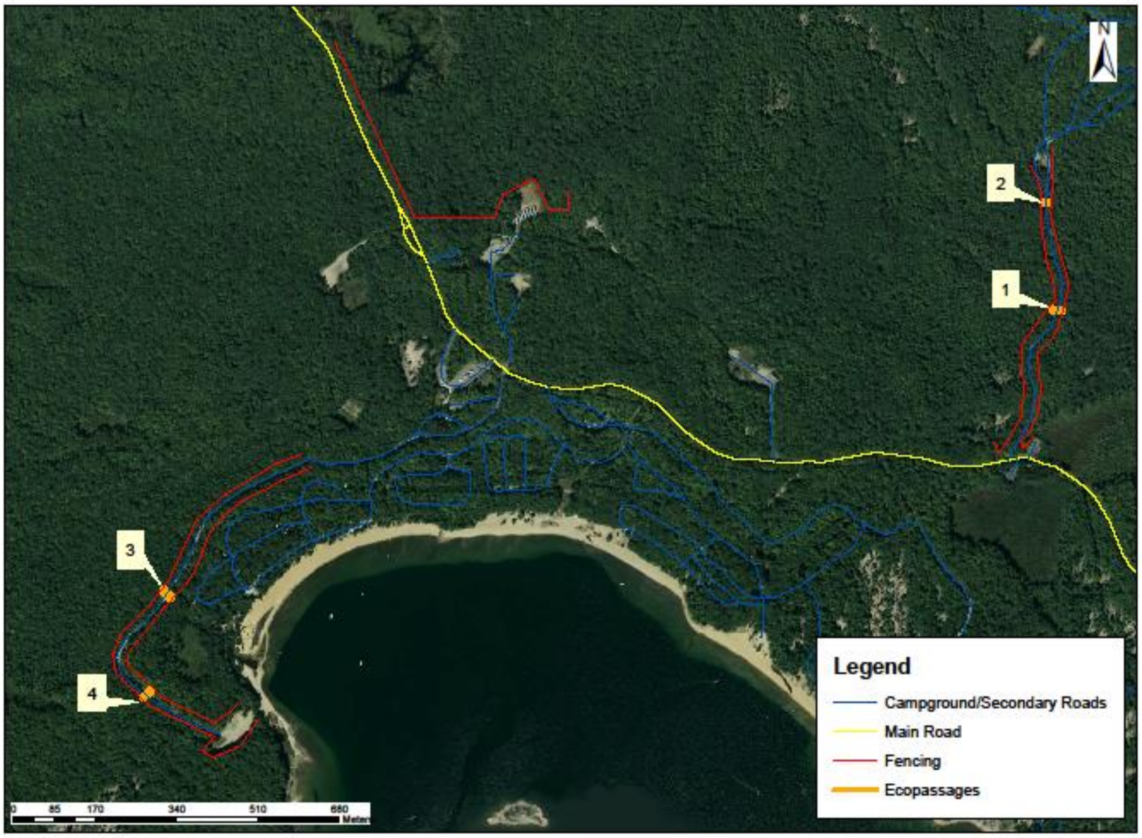

Figure 1.1: Map of Killbear Provincial Park, including mitigation fencing and ecopassages 1 and 2 on Blind Bay Road and ecopassages 3 and 4 on Day Use Road.

Figure 1.2: Brake for Snake Signs installed along roads with known reptile road mortality in Killbear Provincial Park.

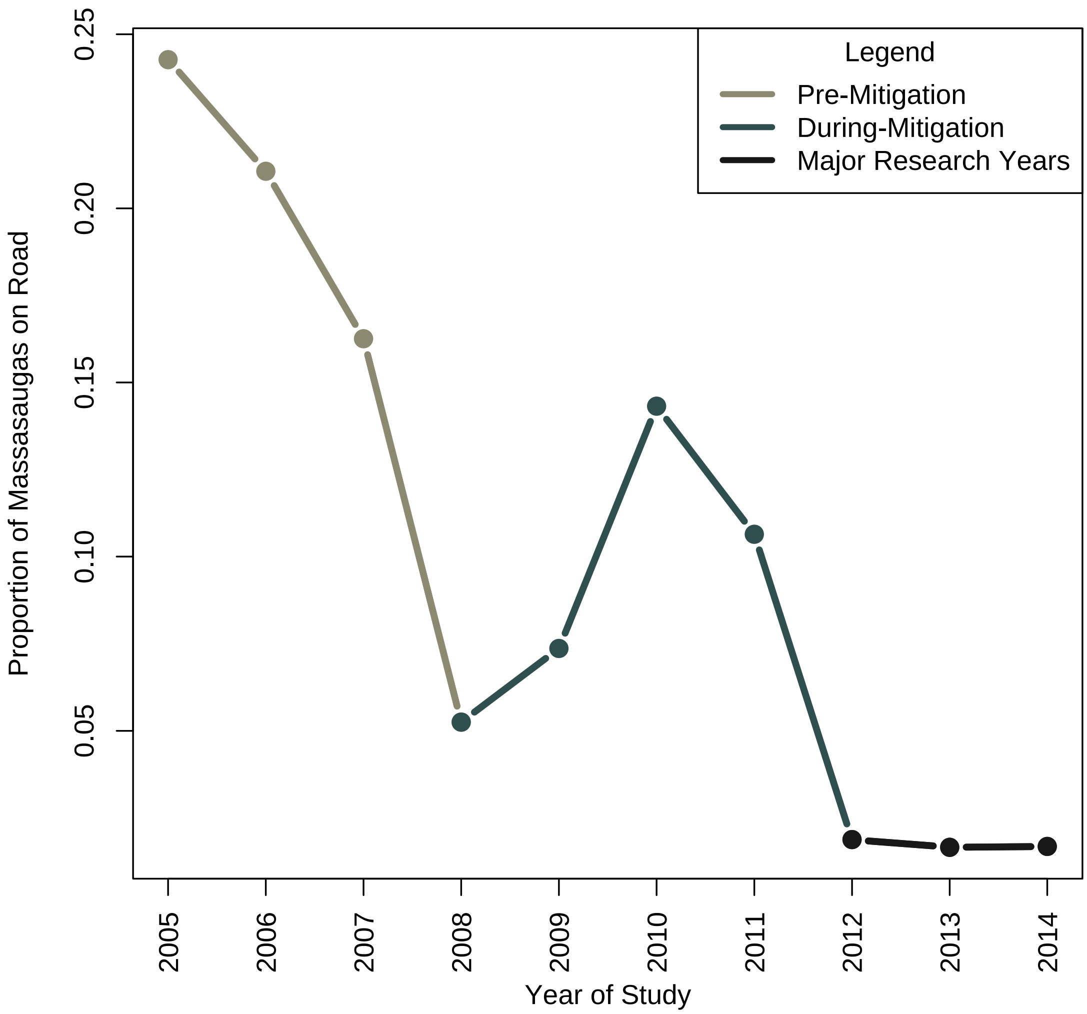

Figure 1.3: Proportion of Massasauga (*Sistrurus catenatus*) captures in Killbear Park that were found dead and alive on road on park survey route (Standard Error=0.026 of proportion of Massasauga captures). The different coloured lines indicate phases of mitigation construction.

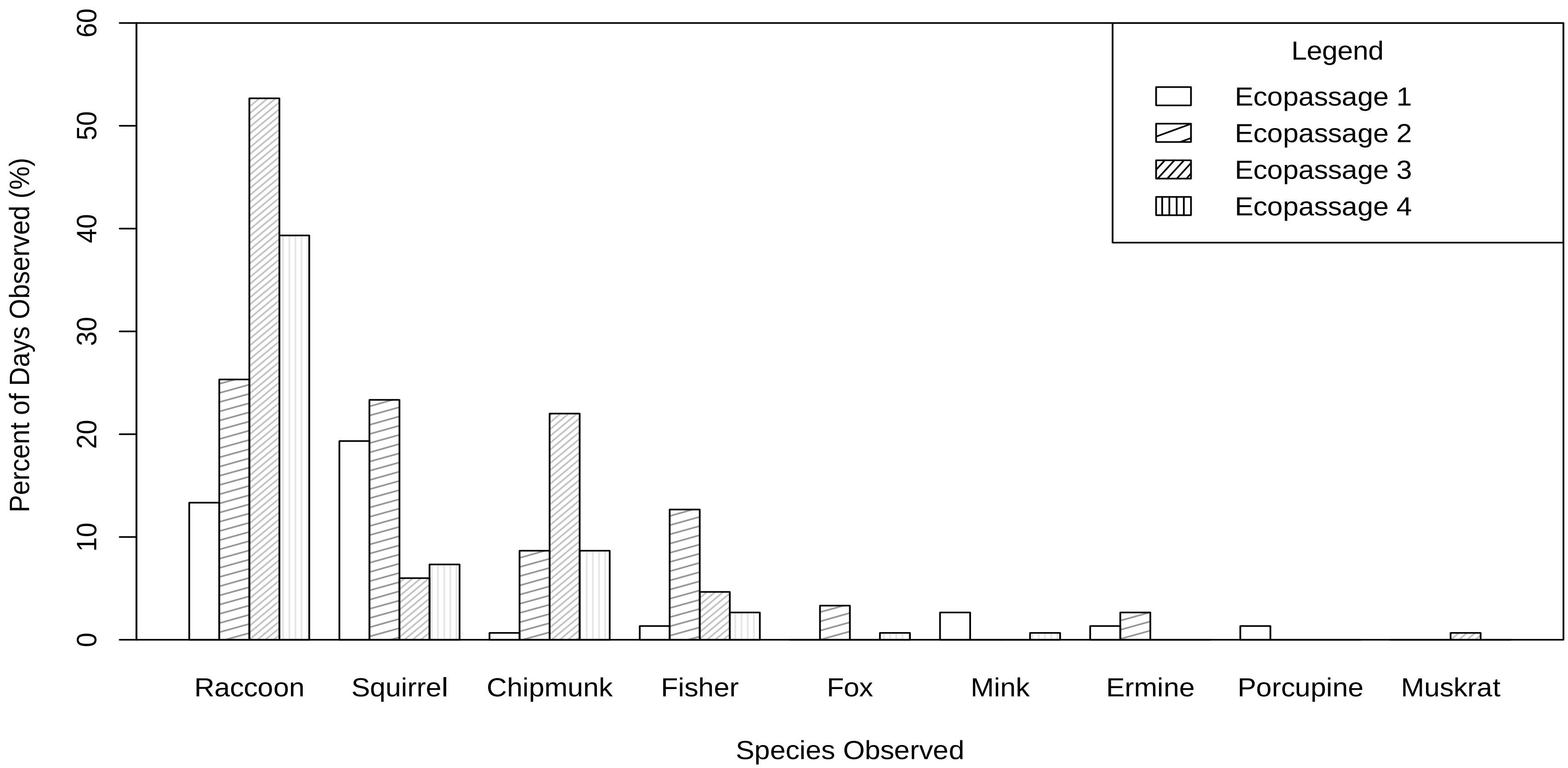

Figure 1.4: Percentage of days out of total days of observation that mammal species were detected by wildlife cameras in Killbear Provincial Park Ecopassages 1-4 during the 2013 field season.

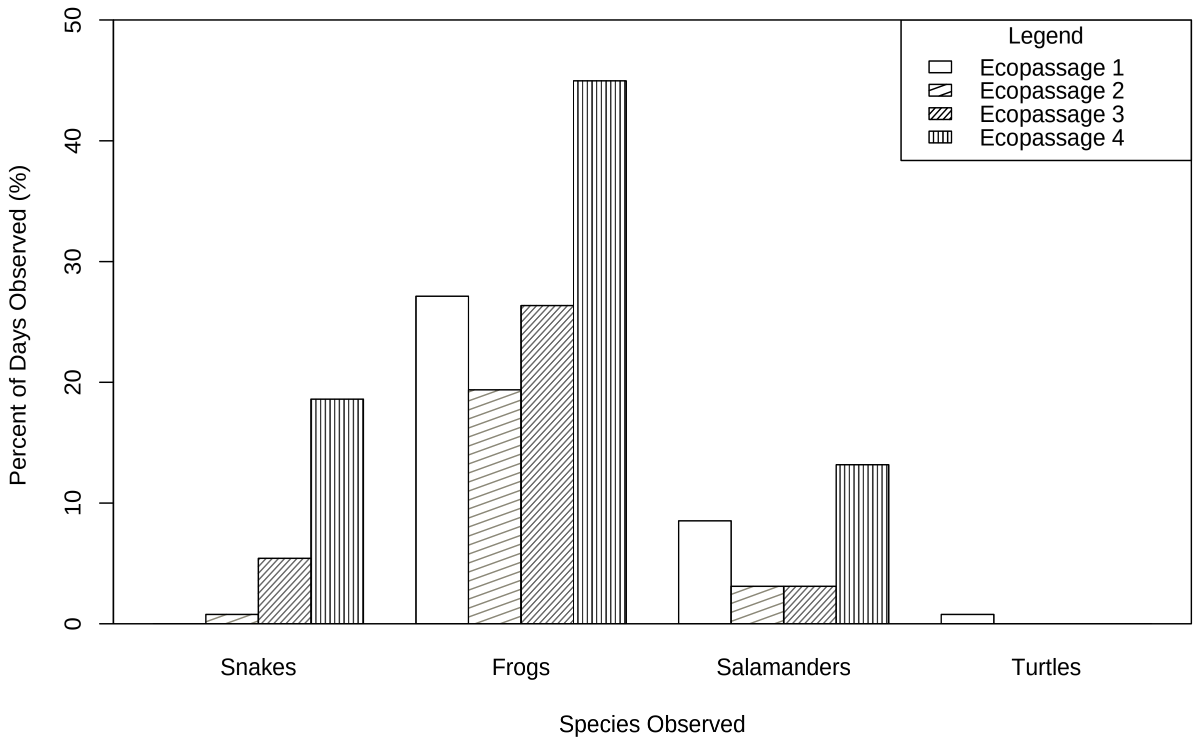

Figure 1.5: Percentage of days that reptiles and amphibians were detected by wildlife cameras in Killbear Provincial Park Ecopassages 1-4 during the 2014 field season.

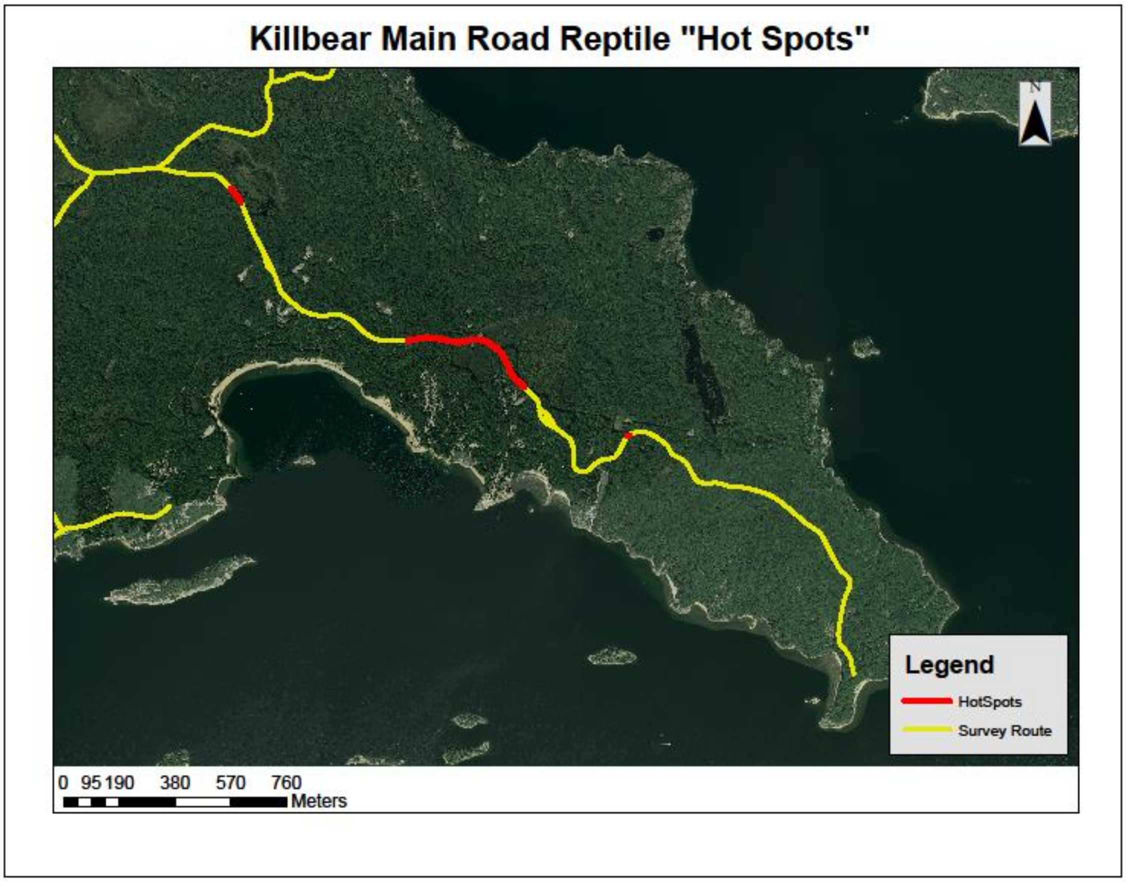

Figure 1.6A: Hot spot analysis of 2013/2014 reptile road mortality on Park Main Road in Killbear Provincial Park, Ontario. Hot spots are indicated in red.

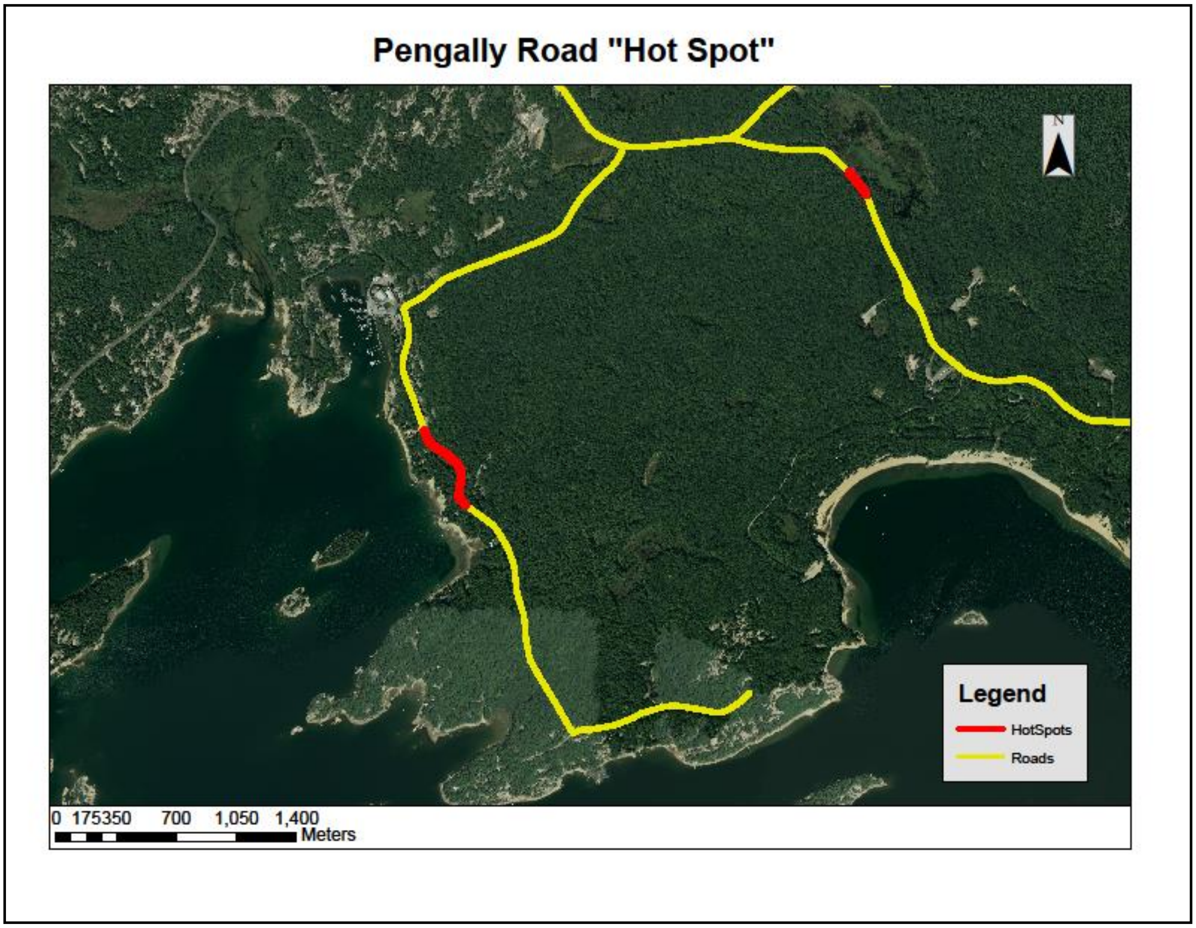

Figure 1.6B: Hot spot analysis of 2013/2014 reptile road mortality on Pengally Road, just outside of Killbear Provincial Park, Ontario. Hot spots are indicated in red.

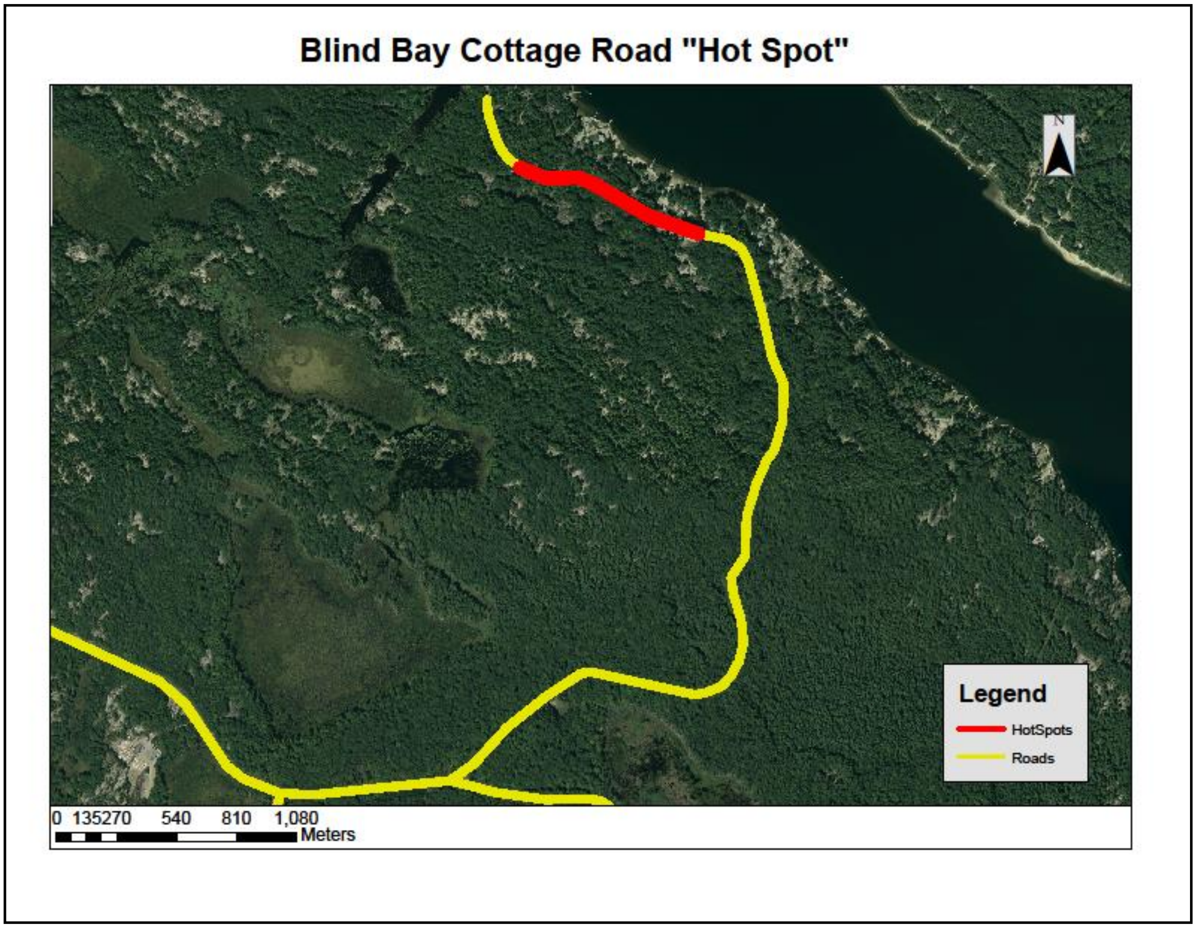

Figure 1.6C: Hot spot analysis of 2013/2014 reptile road mortality on Blind Bay cottage Road. Hot spots are indicated in red.

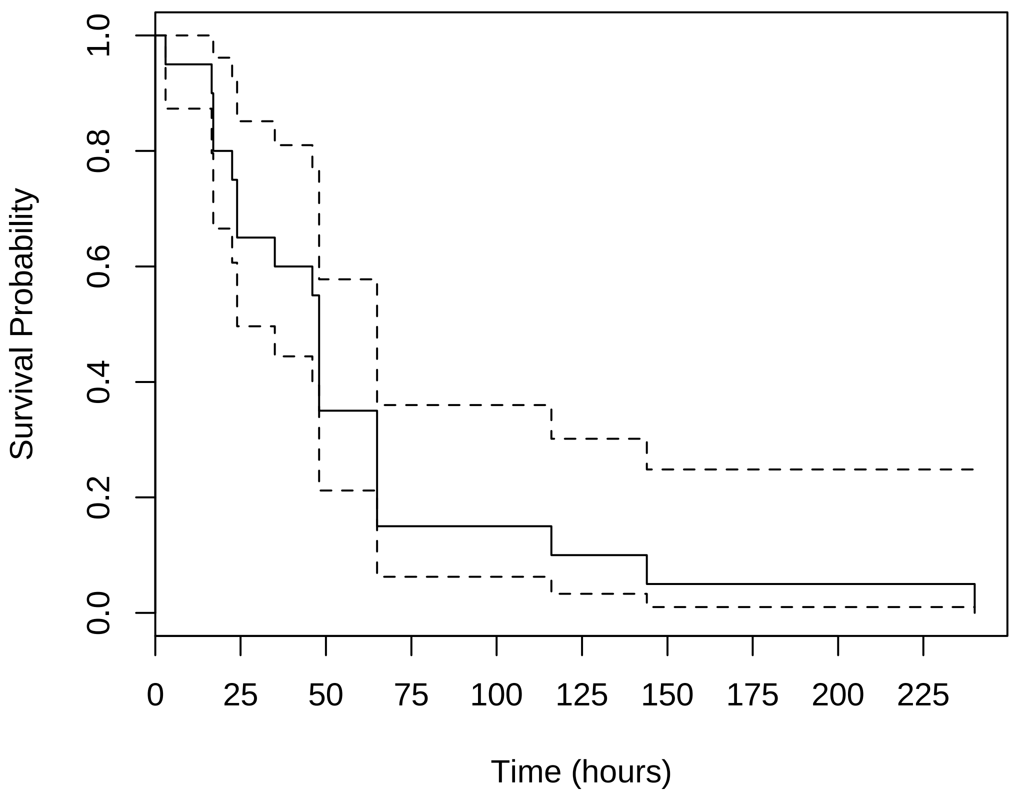

Figure 1.7: The probability that a snake carcass on a road will persist over time before being removed by a scavenger. Survival curve is for Garter snake (*Thamnophis sirtalis*) carcasses on a road. (Kaplan-Meier estimate and corresponding 90% confidence intervals; N=20)

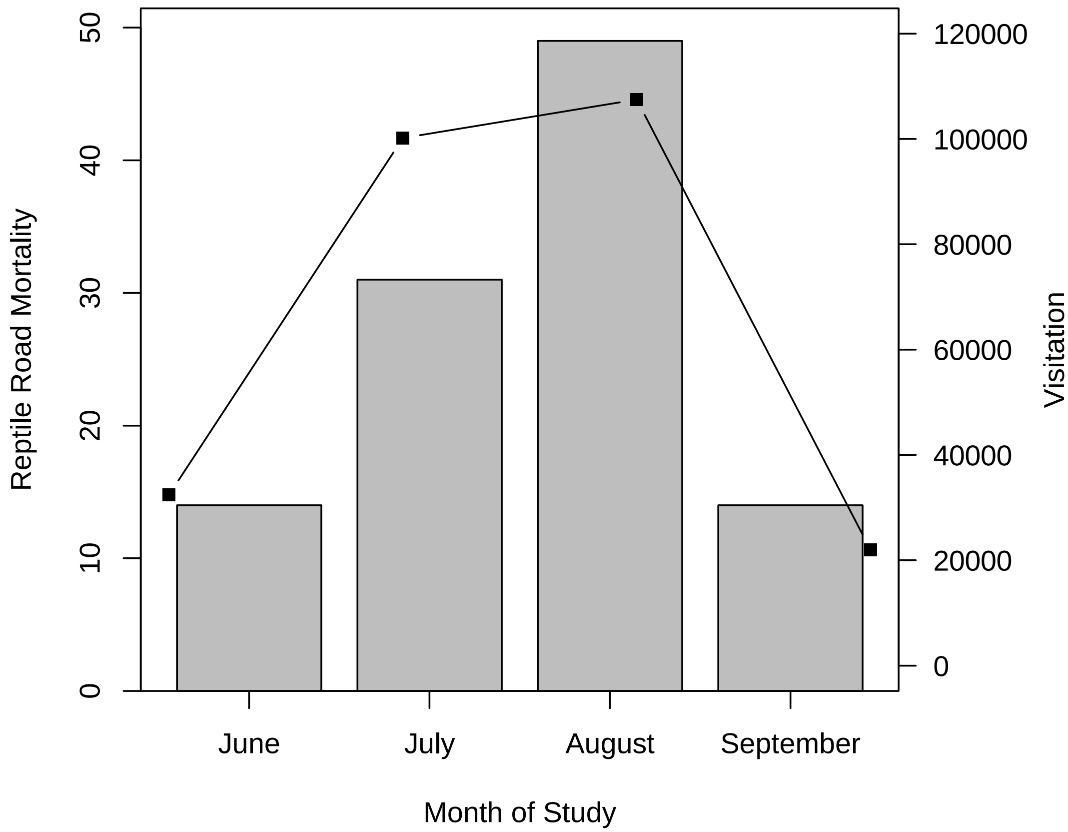

Figure 1.8: Monthly frequencies (number) of reptile road mortality (bars) along with mean monthly park visitation numbers for 2013 and 2014 (line) in Killbear Provincial Park, Ontario.

Appendix 1.1: Results of *post hoc* Tukey tests comparing temperatures inside ecopassage 1, and the surrounding environment (road, forest, entrance).

Month	Time Period	iButton Locations	p-value
June	Morning	Entrance - Ecopassage	0.8990596
June	Morning	Forest - Ecopassage	0.8967798
June	Morning	Road - Ecopassage	**0.0000661**
June	Afternoon	Entrance - Ecopassage	0.5460823
June	Afternoon	Forest - Ecopassage	**$<1.0\text{x}10^{-8}$**
June	Afternoon	Road - Ecopassage	**$<1.0\text{x}10^{-8}$**
June	Night	Entrance - Ecopassage	**0.0000689**
June	Night	Forest - Ecopassage	**$<1.0\text{x}10^{-8}$**
June	Night	Road - Ecopassage	**0.0005606**
July	Morning	Entrance - Ecopassage	**0.0079504**
July	Morning	Forest - Ecopassage	0.7400681
July	Morning	Road - Ecopassage	0.4194673
July	Afternoon	Entrance - Ecopassage	0.7232018
July	Afternoon	Forest - Ecopassage	**$<1.0\text{x}10^{-8}$**
July	Afternoon	Road - Ecopassage	**0.0000052**
July	Night	Entrance - Ecopassage	**$<1.0\text{x}10^{-8}$**
July	Night	Forest - Ecopassage	**$<1.0\text{x}10^{-8}$**
July	Night	Road - Ecopassage	0.9738896
August	Morning	Entrance - Ecopassage	0.7505496
August	Morning	Forest - Ecopassage	0.3291899
August	Morning	Road - Ecopassage	0.2483671
August	Afternoon	Entrance - Ecopassage	**0.0000002**
August	Afternoon	Forest - Ecopassage	**0.0095801**
August	Afternoon	Road - Ecopassage	**$<1.0\text{x}10^{-8}$**
August	Night	Entrance - Ecopassage	**0.0000001**
August	Night	Forest - Ecopassage	**$<1.0\text{x}10^{-8}$**
August	Night	Road - Ecopassage	**0.0248955**
September	Morning	Entrance - Ecopassage	0.9243148
September	Morning	Forest - Ecopassage	0.1078629
September	Morning	Road - Ecopassage	0.9999996
September	Afternoon	Entrance - Ecopassage	**0.0009708**
September	Afternoon	Forest - Ecopassage	0.917118
September	Afternoon	Road - Ecopassage	**0.0000002**
September	Night	Entrance - Ecopassage	0.8655355
September	Night	Forest - Ecopassage	0.9906651
September	Night	Road - Ecopassage	0.9984636

Appendix 1.2: Results of *post hoc* Tukey tests comparing temperatures inside ecopassage 2, and the surrounding environment (road, forest, entrance).

Month	Time Period	iButton Locations	p-value
June	Morning	Entrance - Ecopassage	**0.0012121**
June	Morning	Forest - Ecopassage	**0.0000011**
June	Morning	Road - Ecopassage	0.0999272
June	Afternoon	Entrance - Ecopassage	**$<1.0x10^{-8}$**
June	Afternoon	Forest - Ecopassage	**$<1.0x10^{-8}$**
June	Afternoon	Road - Ecopassage	**$<1.0x10^{-8}$**
June	Night	Entrance - Ecopassage	**0.0005775**
June	Night	Forest - Ecopassage	**$<1.0x10^{-8}$**
June	Night	Road - Ecopassage	**$<1.0x10^{-8}$**
July	Morning	Entrance - Ecopassage	**0.0005262**
July	Morning	Forest - Ecopassage	**0.0000005**
July	Morning	Road - Ecopassage	0.1675536
July	Afternoon	Entrance - Ecopassage	**$<1.0x10^{-8}$**
July	Afternoon	Forest - Ecopassage	**$<1.0x10^{-8}$**
July	Afternoon	Road - Ecopassage	**$<1.0x10^{-8}$**
July	Night	Entrance - Ecopassage	**$<1.0x10^{-8}$**
July	Night	Forest - Ecopassage	**$<1.0x10^{-8}$**
July	Night	Road - Ecopassage	0.9998121
August	Morning	Entrance - Ecopassage	0.2021573
August	Morning	Forest - Ecopassage	0.7277207
August	Morning	Road - Ecopassage	0.6603035
August	Afternoon	Entrance - Ecopassage	**$<1.0x10^{-8}$**
August	Afternoon	Forest - Ecopassage	**0.0018166**
August	Afternoon	Road - Ecopassage	**$<1.0x10^{-8}$**
August	Night	Entrance - Ecopassage	**$<1.0x10^{-8}$**
August	Night	Forest - Ecopassage	**0.0000318**
August	Night	Road - Ecopassage	0.9359159
September	Morning	Entrance - Ecopassage	0.999999
September	Morning	Forest - Ecopassage	0.6731074
September	Morning	Road - Ecopassage	0.6771858
September	Afternoon	Entrance - Ecopassage	**$<1.0x10^{-8}$**
September	Afternoon	Forest - Ecopassage	0.2180812
September	Afternoon	Road - Ecopassage	**$<1.0x10^{-8}$**
September	Night	Entrance - Ecopassage	**0.0001014**
September	Night	Forest - Ecopassage	0.6353023
September	Night	Road - Ecopassage	0.5772962

Appendix 1.3: Results of *post hoc* Tukey tests comparing temperatures inside ecopassage 3, and the surrounding environment (road, forest, entrance).

Month	Time Period	iButton Locations	p-value
June	Morning	Entrance - Ecopassage	**$<1.0x10^{-8}$**
June	Morning	Forest - Ecopassage	**0.0003352**
June	Morning	Road - Ecopassage	**$<1.0x10^{-8}$**
June	Afternoon	Entrance - Ecopassage	**$<1.0x10^{-8}$**
June	Afternoon	Forest - Ecopassage	**0.0000279**
June	Afternoon	Road - Ecopassage	**$<1.0x10^{-8}$**
June	Night	Entrance - Ecopassage	**0.0000013**
June	Night	Forest - Ecopassage	0.5308196
June	Night	Road - Ecopassage	**$<1.0x10^{-8}$**
July	Morning	Entrance - Ecopassage	**0.0000003**
July	Morning	Forest - Ecopassage	0.865056
July	Morning	Road - Ecopassage	**0.00039**
July	Afternoon	Entrance - Ecopassage	**$<1.0x10^{-8}$**
July	Afternoon	Forest - Ecopassage	**0.0000001**
July	Afternoon	Road - Ecopassage	**$<1.0x10^{-8}$**
July	Night	Entrance - Ecopassage	0.6294774
July	Night	Forest - Ecopassage	0.1673614
July	Night	Road - Ecopassage	**0.0000006**
August	Morning	Entrance - Ecopassage	0.3474912
August	Morning	Forest - Ecopassage	0.3237099
August	Morning	Road - Ecopassage	**$<1.0x10^{-8}$**
August	Afternoon	Entrance - Ecopassage	**$<1.0x10^{-8}$**
August	Afternoon	Forest - Ecopassage	**0.0000089**
August	Afternoon	Road - Ecopassage	**$<1.0x10^{-8}$**
August	Night	Entrance - Ecopassage	0.4403307
August	Night	Forest - Ecopassage	**0.0374083**
August	Night	Road - Ecopassage	**$<1.0x10^{-8}$**
September	Morning	Entrance - Ecopassage	0.7328036
September	Morning	Forest - Ecopassage	**0.0698108**
September	Morning	Road - Ecopassage	0.0841004
September	Afternoon	Entrance - Ecopassage	**$<1.0x10^{-8}$**
September	Afternoon	Forest - Ecopassage	**0.0001228**
September	Afternoon	Road - Ecopassage	**$<1.0x10^{-8}$**
September	Night	Entrance - Ecopassage	**0.0000183**
September	Night	Forest - Ecopassage	0.3568133
September	Night	Road - Ecopassage	**0.0229825**

Appendix 1.4: Results of *post hoc* Tukey tests comparing temperatures inside ecopassage 4, and the surrounding environment (road, forest, entrance).

Month	Time Period	iButton Locations	p-value
June	Morning	Entrance - Ecopassage	0.5230062
June	Morning	Forest - Ecopassage	**0.0000425**
June	Morning	Road - Ecopassage	**$<1.0 \times 10^{-8}$**
June	Afternoon	Entrance - Ecopassage	**0.0000213**
June	Afternoon	Forest - Ecopassage	**0.0003943**
June	Afternoon	Road - Ecopassage	**$<1.0 \times 10^{-8}$**
June	Night	Entrance - Ecopassage	**0.0305736**
June	Night	Forest - Ecopassage	**0.0163855**
June	Night	Road - Ecopassage	**$<1.0 \times 10^{-8}$**
July	Morning	Entrance - Ecopassage	0.9971453
July	Morning	Forest - Ecopassage	**0.001384**
July	Morning	Road - Ecopassage	**$<1.0 \times 10^{-8}$**
July	Afternoon	Entrance - Ecopassage	**0.0000009**
July	Afternoon	Forest - Ecopassage	**0.0000018**
July	Afternoon	Road - Ecopassage	**$<1.0 \times 10^{-8}$**
July	Night	Entrance - Ecopassage	0.9999934
July	Night	Forest - Ecopassage	0.7587558
July	Night	Road - Ecopassage	**$<1.0 \times 10^{-8}$**
August	Morning	Entrance - Ecopassage	0.1985145
August	Morning	Forest - Ecopassage	0.7977801
August	Morning	Road - Ecopassage	**0.0566862**
August	Afternoon	Entrance - Ecopassage	**$<1.0 \times 10^{-8}$**
August	Afternoon	Forest - Ecopassage	**$<1.0 \times 10^{-8}$**
August	Afternoon	Road - Ecopassage	**$<1.0 \times 10^{-8}$**
August	Night	Entrance - Ecopassage	0.1289298
August	Night	Forest - Ecopassage	0.6394472
August	Night	Road - Ecopassage	**$<1.0 \times 10^{-8}$**
September	Morning	Entrance - Ecopassage	0.0622376
September	Morning	Forest - Ecopassage	**0.037367**
September	Morning	Road - Ecopassage	0.1264045
September	Afternoon	Entrance - Ecopassage	**$<1.0 \times 10^{-8}$**
September	Afternoon	Forest - Ecopassage	**0.0000007**
September	Afternoon	Road - Ecopassage	**$<1.0 \times 10^{-8}$**
September	Night	Entrance - Ecopassage	**0.0097077**
September	Night	Forest - Ecopassage	0.1198849
September	Night	Road - Ecopassage	**0.0018042**

Chapter 2

Population Viability Analysis:

The importance of road mortality mitigation for preventing extirpation of a Massasauga Rattlesnake population in a protected area

Abstract

Snake populations are declining globally due to a range of factors that includes road mortality. Monitoring post-construction of road mortality mitigation measures has become more common, but few of these assessments look at the long-term effect on population viability. Killbear Provincial Park, Ontario recognized the damaging effects of road mortality on local snakes and began monitoring populations of the threatened Eastern Massasauga rattlesnake (*Sistrurus catenatus*) in 1992. This work resulted in a long-term database of Massasauga captures (1992-present). To address concerns of potentially declining Massasauga populations, Killbear installed barrier fencing and 4 ecopassages along 3 busy park roads between 2007 and 2013. I did a population viability analysis (Vortex) using local demographic data and road mortality rates to determine if mitigation installed in Killbear enhances Massasauga population persistence into the future with low risk of extinction. I found that had mitigation not been constructed, the study population of Massasaugas would have sustained high levels of road mortality, resulting in an almost 100% probability of extinction over 100 years. I found that the post-mitigation road mortality levels corresponded with a low probability of extinction, suggesting that the mitigation is effective at promoting a sustainable population. However, the population still appears highly sensitive to additive female mortalities, and the death of more than 2 females per year could result in an extinction risk greater than 85%. Therefore the road mortality rates should be revisited over time to ensure that future levels of road mortality do not fluctuate. Analyzing the effects of road mortality at a population level helps ensure proper management action can be taken to increase its long-term viability.

Introduction

Snake populations are declining globally due to a range of factors that includes road mortality (Böhme et al. 2013). To preserve populations, we must properly assess these species' needs for a viable future (Reading et al. 2010). An important goal of species at risk management is to make reliable population size estimates, to allow biologists to determine whether a species is in decline and facing risk of extinction (Mace & Lande 1991; Seigel & Sheil 1999; COSEWIC 2011; IUCN 2012). Modelling snake population trends can be challenging; fortunately there are population modeling techniques available to biologists that facilitate the assessment of population trends, including population viability analysis (PVA – Parker and Plummer 1987; Possingham et al. 1993; Akcakaya & Sjogren-Golve 2000; Patterson & Murray 2008; Mullin & Seigel 2009).

Assessing population viability is increasingly important in conservation planning (Akcakaya & Sjogren-Golve 2000). A PVA can provide valuable insights such as the likelihood of a species' decline/extinction (Beissinger & Westphal 1998; Akcakaya & Sjogren-Golve 2000). Conservation groups such as the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and the International Union for Conservation of Nature (IUCN) use quantitative analyses such as PVA to assess the status and designation of focal species. PVA has also become a popular tool for biologists and has been applied to a variety of taxa, including birds, mammals, and reptiles (Crouse et al. 1987; Seigel & Sheil 1999; Row et al. 2007; Enneson & Litzgus 2008), although it has rarely been used for snakes (Middleton & Chu 2004; Breininger et al. 2012; Hyslop et al. 2012).

PVAs have also become important for analyzing populations of species that have been reduced in size due to road kill (Seigel & Sheil 1999; Row et al. 2007). Road mortality reduces population viability and can eventually cause the extinction of a species (Fahrig et al. 1995; Gibbs & Shriver 2002). PVAs can determine the specific effects that road mortality has on the population demographics of focal species and most importantly, species that are at risk (Row et al. 2007). PVAs have been used for the analysis of several Massasauga populations in Ontario, including those at Georgian Bay Islands National Park, Wainfleet Bog, Ojibway Prairie and the Bruce Peninsula (Middleton & Chu 2004), but not in Killbear where conservation actions have been implemented to specifically mitigate road mortality.

The life history traits of the Massasauga make it difficult to observe demographic trends in populations without long-term mark-recapture studies (Seigel & Sheil 1999). Although long-term studies on most snake populations are lacking (Reading et al. 2010), Killbear Provincial Park has been collecting data on the Massasauga since 1992. I used this long-term database, along with data collected during my two-year study, past Massasauga PVAs in Ontario, and published literature, to inform a PVA to determine the status of the Massasauga population in Killbear, and to assess the effects that road mortality and mitigation have had on population demographics.

I deemed the level of mitigation to be adequate if my PVA results suggested that post-mitigation road mortality levels allowed for a higher probability of population persistence of Massasaugas in Killbear than before mitigation occurred. I also explored further mitigation options that would ensure a minimal viable population size is achieved. Finally, I examined the effects that road mortality would have had on Massasauga populations in Killbear had mitigation not taken place, further contributing to the evaluation of the effectiveness of road mortality mitigation measures.

Methods

Study area

There are four disjunct populations of Massasaugas in Ontario, with one of the largest occurring on the eastern shore of Georgian Bay (Parent & Weatherhead 2000). Killbear Provincial Park, located in Carling Township, Parry Sound District, Ontario holds part of this population. There are two subpopulations of Massasaugas in Killbear: Population A and Population B (Rouse 2005). These two subpopulations rarely interact and are separated by geographical and anthropogenic barriers. My study focused only on Population A because this population has been well studied in comparison to Population B (Rouse 2005; K. Otterbein pers. comm. 2013). Massasauga research has been conducted in Killbear since 1992 (Parent & Weatherhead 1998, 2000), resulting in a long-term database, but a lack of search effort and a possible decline in numbers in recent years has resulted in insufficient data (recaptures) to accurately produce a population estimate for Population B (K. Otterbein pers. comm. 2013).

Road mortality of Massasaugas in the park sparked concern for the population, which prompted the construction of mitigation. Mitigation measures were installed in Killbear from 2007-2013, including barrier fencing on three different roads totaling 3 km and the construction of four ecopassages on two separate roads (Chapter 1).

Population Viability Analysis

Previous population viability analyses (Seigel & Sheil 1999; Middleton & Chu 2004) provided demographic data to inform modelling and to allow inter-population comparisons. Seigel and Sheil (1999) conducted a preliminary PVA of a Massasauga population in Missouri in 1999. In Ontario, PVAs have been done for Georgian Bay Island National Park, Bruce Peninsula, and Wainfleet Bog by Middleton and Chu (2004), Bruce Peninsula by Miller (2005) and Ojibway Prairie by Brennan (2004). These previous studies provide context for my PVA in Killbear for which long-term demographic data are available.

There are three standard PVA software packages discussed by Middleton and Chu (2004) including: RAMAS-metapop, Vortex, and ALEX-FREE. Each software package has relatively similar capabilities and produces comparable results (Middleton & Chu 2004). I used Vortex version 10.0.7.9 (Lacy & Pollak 2014) because this software is provided at no cost, allowing future research to update this PVA without concern for financial constraints. Vortex is a Monte Carlo simulation of the effects of a variety of parameters on the population being studied (Miller 2005, Lacey et al. 2015). It models a population in specific sequential events based on biological constants and includes stochasticity through a life cycle (Lacy 1993, Miller 2005, Lacy et al. 2015). Vortex does not require the user to create models or matrixes, but rather the user inputs biological parameters into a general user interface (Lacy et al. 2015).

Input Parameters

PVA software packages offer a wide selection of input parameters to account for a range of biological factors. There is no standardized set of PVA parameters that can be applied to every situation meaning that each study is unique (Akcakaya & Sjogren-Golve 2000). All major biological factors that could be accurately estimated from Killbear's dataset, past PVAs, and published literature were used to parameterize my PVA. Parameters that could not be reliably extrapolated or estimated were not used to avoid the risk of producing inaccurate results that would have limited ecological applicability to the Killbear population. This conservative approach was also used by Middleton and Chu (2004) for several other populations of Massasaugas in Ontario.

To produce output comparable with other Massasauga populations in Ontario, my study focused on the same parameters that were used for PVAs of populations in Georgian Bay Islands National Park, Ojibway Prairie, Wainfleet Bog and the Bruce Peninsula. This ensured that the PVA for Killbear produced results that could be considered suitable for conservation purposes in Ontario. The parameters used for the PVA are summarized in Table 2.1 and explained below:

Carrying Capacity

Determining the true carrying capacity of Massasaugas in the wild is difficult and little has been published on this topic (Miller 2005). To minimize unknown biological constraints and to be consistent with other Ontario PVAs, I considered the Killbear population to have an unlimited carrying capacity (set at 10 000 individuals).

Quasi-extinction Threshold

A population that falls below a certain size can be considered functionally extinct (Ellner et al. 2002, Miller 2005). As populations decline, a certain point will be reached where biological factors combine resulting in the population no longer being recoverable (Miller 2005). Similar to past studies, I have set this point at 10 individuals; therefore extinction risks reported are indicative of the population falling below this threshold as opposed to corresponding with a population size of zero (Ellner et al. 2002, Middleton & Chu 2004, Brennan 2004, Miller 2005).

Breeding System

Massasaugas have a polygamous mating system (Jellen et al. 2007). There are no data indicating multiple yearly matings by male Massasaugas, but such behaviour has been observed in other vipers (Schuett & Gillingham 1986); therefore in my model, males were randomly paired with a given female for breeding, and were returned to the pool to possibly breed again within the same year (Miller 2005, Jellen et al. 2007). All adult males in the Killbear population were considered available to breed every year.

Breeding Frequency

Female Massasauga breeding frequency varies based on geographic location: breeding is annual in the southern part of the species' range and biennial in the northern part of the range (Howard 1981, Szymanski 1998, Parker & Prior 1999, Middleton & Chu 2004, Miller 2005, Aldridge et al. 2008). Due to a short growing season in Parry Sound District, several studies have found biannual reproduction in female Massasaugas, a trend that has been well-documented in Killbear (Rouse 2005). This pattern does not suggest that all Massasaugas females mate in one given year, but rather a different subpopulation of females will be gravid each year (Parker & Prior 1999). Therefore the breeding frequency was set at 0.50.

Sex Ratio

Past Ontario PVAs assumed a sex ratio of one adult female to one adult male snake (Brennan 2004, Middleton & Chu 2004). Capture records for Killbear indicate a female-biased population, but this is most likely due to the skewed probability of observing females during surveys. Killbear staff have concentrated search effort on gestation sites resulting in higher female encounters (Rouse 2005). Male Massasaugas spend more time in wetlands and travel more than females, and therefore are not encountered as often but are also more likely to encounter dangerous situations while searching for females (Rouse 2005, Rouse et al. 2011, K. Otterbein pers. comm. 2015). Depending on the timing and location of sampling, apparent sex ratios may vary, but generally do not significantly differ from 1:1, and for simplicity and consistency I used a 1:1 sex ratio in my analysis (Johnson 1995, Seigel & Sheil 1999).

Average Litter Size

Litter size of Massasaugas can range from 9-19 neonates (Aldridge et al. 2008). The average litter size across the species' range is 9.3, but mean litter size varies among locations (Aldridge et al. 2008). An intensive analysis of Killbear's long-term database was completed in 1999 for the Killbear Massasauga Management plan, and it was determined that the average litter size was 10.7 neonates; therefore this number was used for my PVA (Rouse 2005, K. Otterbein pers. comm. 2015). A 1:1 sex ratio of neonates was used.

Age of First Reproduction

The age of first reproduction refers to the age at first birth not sexual maturity or first conception per se (Lacy & Pollak 2014). Ontario PVAs have assumed that Massasauga age at first reproduction is 6 (Middleton & Chu 2004, Miller 2005). Parent and Weatherhead (2000) determined through long-term monitoring of individual Massasaugas in Killbear that the majority of snakes reached sexual maturity at 4-5 years of age and gave birth to their first litter at 5-6 years of age. Consistent with Middleton and Chu (2004) and Miller (2005), 6 years of age was used as the age of first reproduction in my analysis, for both males and females.

Initial Population Size

Mark-recapture censuses were conducted in Killbear from 1992 to 2014 in various capacities. Killbear staff and researchers conducted yearly abundance surveys in known Massasauga locations. Mark-recapture census data also include opportunistic captures on campsites, cottages and roads. I used the dataset from 2011 to 2014 to estimate abundance because of the large sample size and constant search effort compared to previous years. The study area was relatively constant from 2011 to 2014 and did not vary as much as in previous years. Constant study area is an important assumption needed for population modelling and results will not be accurate if violated (Cooch & White 2015).

Population abundance of adults (individuals > 126 g; Rouse 1999) was estimated using a Cormack-Jolly-Seber model based on the POPAN option in Program MARK (White & Burnham 1999). Not all records in the dataset indicated the sex of individual snakes; therefore the population abundance estimate was conducted for adult females and males as one group. The POPAN model makes use of apparent survival (phi), capture probability (p), probability of entry (pent) and population size (N) (Cooch & White 2015). I fitted six POPAN models, which were constructed from combinations of fully time-dependent and constant parameters (survival, capture, entry, population size).

Parameter-specific link functions were defined prior to running the models. The *sin* link function was specified for both survival and recapture parameters, the multinomial *logit* link function was specified for the entry parameter, and the *log* function was specified for the super-population size (all animals never captured). Models were compared using Quasi-Akaike's Information Criterion (Cooch & White 2015). Using the program RELEASE, I did goodness of fit tests (Chi-square) to check for over-dispersion. Using the population size estimate of adult snakes, life history characteristics for females, and survival rates of age classes, I estimated the number of juveniles in the population.

Mortality

Data on mortality rates for neonate and juvenile snakes are often lacking due to the difficulty of observing them in the field. This observer bias results in inaccurate age-specific mortality rates of younger snakes. To be consistent, I based the mortality rates of neonates and juveniles on the estimates provided by Miller (2005) for the Bruce Peninsula population. Adult mortality rates can be accurately estimated, if long-term data are available for a given site. I used an annual adult mortality rate of 28% for the Killbear population as determined by previous radiotelemetry data (Jones et al. 2012). This mortality rate was parsed for human-caused mortalities, allowing road mortality to be accounted for in a sensitivity analysis as opposed to the base model. Adults were defined as having reached sexual maturity. Six age classes (in years) were used in my study: Neonates (0-1), Juveniles 1 (1-2), Juveniles 2 (2-3), Juveniles 3 (3-4), Juveniles 4 (4-5), and Adults (5+).

Base Model

The base model PVA was simulated 500 times with the set of parameters described above, over a timeframe of 100 years. The "terminal risk of extinction" and the instantaneous exponential growth rate (r) were recorded for each simulated dataset.

Sensitivity Analysis

Sensitivity analysis varies parameters over a range of values to assess their relative importance to the viability of the study population (Middleton & Chu 2004). This tool provides insights on the relative estimates of risks involved with changing parameters, such as incidental mortality (Akcakaya & Sjogren-Golve 2000; Middleton & Chu 2004). After a base population model was simulated, I conducted a series of sensitivity analyses to determine the impact of road mortality and the effects of road mortality mitigation on the demographics of Massasaugas in Killbear (as noted above, the base model mortality rates did not account for road mortality). An external source of mortality (Harvest) was inputted into two sensitivity models: i) post mitigation scenario, and ii) no mitigation scenario. Both scenarios were simulated for each sex of both adults and juveniles. The sex ratio of roadkill in each scenario was varied.

<u>Simulating Road Mortality as a "Harvest"</u>

Massasauga road data were collected during bicycle surveys, car surveys and incidental observations as outlined in Chapter 1. Within my study site, no road mortality occurred in 2013 but two live snakes (two adult males) were found on the road. In 2014 there were five mortalities (two adult males, two juveniles, one unknown) and two live snakes (one adult male, one juvenile) found on the road. The unknown snake could not be confidently sexed as it had deteriorated. Additional Massasauga road mortality was observed in Killbear and the surrounding area during this study, but these mortalities were not part of the study population and therefore not used in the PVA (K. Otterbein pers. comm. 2015).

For scenario (i) post mitigation, Harvest was set as the highest observed annual road mortality post-mitigation (N=5). Snakes found alive on the road were not used, as these snakes were still part of the current population and therefore did not contribute to an increased risk of extinction. For scenario (ii) no mitigation, Harvest was set as the post-mitigation mortality in the first scenario plus the potential number of Massasauga road mortalities had mitigation structures not been constructed. Potential road mortality was calculated as the number of snakes that crossed ecopassages in 2014. Had mitigation not occurred, the snakes crossing the ecopassages would have crossed the road, where they potentially could have been killed. A second (ii) no mitigation scenario was conducted with a lower Harvest value (N=10) to account for the possibility of snakes crossing the road successfully. Harvest took place once each year of the 100 year simulation.

Results

Initial Population

The goodness of fit tests of Program RELEASE (TEST2+TEST3) found mild dispersion in the data (χ^2=4.79, df=4, p=0.31) resulting in a c-hat of 1.2 for the POPAN datasets. The model with constant recaptures and survival was the highest ranking model with a QAICc weighting of 147.7 (Table 2.2). The population models implemented in

MARK (POPAN) estimated a population size of 72.48 adults with a lower 95% confidence limit of 50.43 and an upper limit of 104.44 (SE=13.60).

Base Model

Using the parameters presented in Table 2.2 (i.e., no additive mortality from anthropogenic sources), the quasi-extinction risk for 500 simulations over 100 years was 0%, and the mean instantaneous growth rate (r) over all years was 0.012. Results of the base model simulation are shown in Figure 2.1.

Post-Mitigation Scenario

Analyses based on the addition of 2014 road mortality data suggest that the current Massasauga population in Killbear is experiencing growth rates ranging from 0.012 to -0.146 and extinction probabilities of 0 to 100% (Figure 2.2) depending on the age and sex of snakes being killed on the road. When road mortality of adults (N=5 per year) was included in the base model, extinction risk increased and population growth rates decreased (Table 2.3). Extinction risk and growth rate were very sensitive to the number of additional females killed; the number of additional males killed had less of an effect on the extinction probability.

Compared to adults, juvenile road mortalities resulted in higher growth rates and lower extinction risks. Similar to the adults, extinction risk and population growth rate of juveniles was also sensitive to the number of females killed. The mean time to the first extinction event when all mortalities were adults ranged from 21.9 to 67.3 years, and ranged from 48.3 to 79.0 years when all mortalities were juveniles (Figure 2.3).

No Mitigation Scenario

If no mitigation had been undertaken in Killbear, 14 Massasaugas might have been killed on the road, based on observations of crossing through the ecopassages (Chapter 1). The addition of potential road mortality in 2014 to the base model, suggests that the Massasauga population in Killbear would experience population growth rates of 0.01 to -0.314 (Table 2.4) and extinction risks of 2 to 100% (Figure 2.4) depending on the age and sex of snakes being killed on the road, although the majority of simulations resulted in a 100% extinction risk.

When road mortality of adults (N=14) was added to the base model, all population simulations had a negative growth rate and an extinction risk of 100%. The majority of juvenile simulations also resulted in negative population growth rates and extinction risks of 100%. Juvenile extinction risks were under 100% if four or fewer females were killed on the road and growth rates were positive only if two or fewer females were killed on the road. The mean time to the first extinction event, if all mortalities were adults, ranged from 10.6 to 17.6 years and ranged from 21.6 to 63.5 if all mortalities were juveniles (Figure 2.5).

The lower no mitigation scenario (N=10) suggests that the Massasauga population in Killbear would experience population growth rates of 0.011 to -0.257 (Table 2.5) and extinction risks of 0.5 to 100% (Figure 2.6) depending on the age and sex of snakes being killed on the road, although the majority of simulations resulted in a 100% extinction risk. The mean time to the first extinction event, if all mortalities were adults, ranged from 12.9 to 33.3 years and ranged from 26.2 to 63.5 if all mortalities were juveniles (Figure 2.7).

Discussion

The base model suggests that Population A of Massasaugas in Killbear has the potential to steadily grow if not impacted by road mortality or other anthropogenic effects. Unfortunately the effects associated with roads are a reality in Southern Ontario ecosystems, negatively effecting otherwise potentially stable wildlife populations. The ecological impact of roads has been estimated to extend up to 15-20% of the total land area of most nations (Reijen et al. 1995) and can result in heavy direct mortality of wildlife (Trombulak & Frissell 2000). Longer lived species with delayed sexual maturity, such as the Massasauga (Parent & Weatherhead 2000) are especially vulnerable to road mortality, posing a direct threat to their population's probability of persistence (Brooks et al. 1991, Congdon et al. 1994). Consequently it is important for population modelling to carefully consider the effects of road mortality.

Although the addition of road mortality to Population A caused the population growth rate to decrease, it appears that the Killbear population can withstand a low level of annual road mortality. The level of road mortality that would allow the Killbear population to persist over time depends on the age and sex of snakes being killed. The model was extremely sensitive to adult female mortalities while the number of males killed had little effect on extinction probability. Unfortunately historical data from Killbear does not contain accurate records of the sex of snakes found on the road. The death of five adult males per year would result in an extinction probability of 1%, whereas the mortality of as few as two adult females per year resulted in an extinction probability greater than 85%. Although juvenile mortality had a lesser effect than that of adults, the mortality of female juveniles still had a negative effect and further increased the extinction risk.

These trends have also been documented elsewhere in Ontario. In a study of Gray ratsnakes (*Pantherophis spiloides*), Row et al. (2007) found that populations were extremely sensitive to female mortality, with three adult female road kills per year resulting in greater than 80% extinction risk in 500 years, while the number of males killed had little effect on extinction probability. Miller (2005) determined that an increase in adult mortality of 15% of the Massasauga population in the Bruce Peninsula resulted in an increase to extinction risk by almost 85% in 100 years. These populations were all extremely sensitive to female mortalities while male mortalities had little to no effect on extinction risk. This was also determined to be true for Butler's Gartersnakes (*Thamnophis butleri*) in Wisconsin, USA (Hyde et al. 2007). Hyde et al. (2007) determined that a 5% increase in female Butler's Gartersnake mortality resulted in a 10.6% increase in probability of extinction in 100 years.

Current road mortality levels, post mitigation, appear to not be a threat to the persistence of Population A in Killbear. Given the demographics of road mortality records from 2013 and 2014, populations can persist in the long term so long as the mitigation is maintained. Road mortality records varied greatly between the two study years (N_{2013}=0, N_{2014}=5); therefore it is plausible that mortality rates may change over time resulting in increased extinction probabilities. I thus recommend that Killbear further monitor Population A for road mortality incidents, paying specific attention to the age and sex of snakes killed on the road. If, over time, road mortality trends show frequent adult female deaths (>2), then future mitigation efforts are warranted. If current road kill trends persist, then current mitigation measures should allow for the Massasauga population to persist into the future, and further action need not be taken.

PVAs can be used to determine if the risk faced by a particular species is acceptable (Akcakaya & Sjogren-Golve 2000). Using ecopassage usage rates from Population A, I determined that had mitigation not been undertaken, the population would have had a diminished probability of persistence. Regardless of sex, extinction would be highly probable if all the snakes using the ecopassage had crossed the road and been killed. Given this trend, I conclude that current mitigation measures will be successful at maintaining a minimum viable population of Massasaugas in the long-term.

I also recommend that the park focus future studies on Population B, which was not included in this study because of low sample size. This subpopulation has suffered from high pre-mitigation levels of road mortality, reaching 15 individuals in some years. The Population A model suggests that the mean time to first extinction for populations suffering from similar road mortality is as little as 10-16 years. There have been difficulties in locating snakes in recent surveys of Population B, and ecopassage usage rates of Population B are low compared to those of Population A. Given the possible high risk and short time to extinction for populations suffering this level of road mortality, Population B should be further studied to determine its status and explore possible recovery options.

PVAs are one of the main tools for conservation planning and management as they allow for the integration of knowledge from all available sources (Akcakaya &

Sjogren-Golve 2000). Despite the popularity and usefulness of PVA, there are some difficulties surrounding its use. Much effort and commitment is needed to accurately inform a PVA model (i.e. provide robust, population-specific parameter estimates), as each case will be unique (Akcakaya & Sjogren-Golve 2000). Such constraints and limitations of current data and knowledge mean that it can be challenging to accurately represent the dynamic nature of biological systems (Lacy et al. 2013). To adequately determine the status of any population requires substantial data accrued over many years (Seigel & Shiel 1999, Akcakaya & Sjogren-Golve 2000). Despite difficulties in acquiring sufficient data, PVAs can account for some uncertainties while still providing rigor (Possingham et al. 1993, Akcakaya & Sjogren-Golve 2000). PVAs also are invaluable in providing a framework for assembling data and thus focusing future research by highlighting gaps in existing data (Possingham 1993).

It is good practice to interpret PVA results with considerable caution. Like other studies, I took a conservative approach when deciding on values for parameters (Brennan 2004, Aldridge et al. 2008). Complete data are not always available, and to reduce the complexity in my analyses, I ignored Allee Effects, spatial effects and genetic consequences of small populations. My goal was to conduct a PVA that would represent the Killbear Massasauga population using the best data available and that would provide something of immediate conservation utility. Absolute numbers should be viewed with care, and instead overarching trends caused by the effects of changing parameters should guide conservation decisions. Each individual PVA represents what we know at a particular time (Middleton & Chu 2004). New data should be added as they become available and as changes occur to populations or habitats due to natural or anthropogenic causes.

Conclusion

My modelling of Population A of Massasaugas in Killbear implies that the road mortality in the last two years, post mitigation, does not pose a threat to population persistence, suggesting that barrier fencing and ecopassages are working well. The population appears to be very sensitive to female mortalities; therefore this model should be revisited over time to ensure that future levels of road mortality allow for maintenance of a minimum viable population of Massasaugas in Killbear. Further, I found that had mitigation (fencing, ecopassages) not been constructed, the Massasauga subpopulation would not be able to withstand the high levels of road mortality. These results strengthen the view that road mortality can have a significant impact on populations of long-lived species (Gibbs & Shriver 2002; Row et al. 2007). If no measures are taken to decrease mortality, it is probable that many populations of long-lived species in close to proximity to roads will be extirpated or experience marked declines.

Road ecology studies should focus on understanding and predicting how roads affect the probability that wildlife populations will persist (Brehme et al. 2013). Many road mitigation structures are not quantitatively evaluated because few have used modeling to assess ecopassage performance. Population modelling ensures a formalized approach to mitigation strategies, which will in turn help to understand the full effects of mitigation efforts on populations now and into the future. A properly conducted PVA ensures that stakeholders can be adequately informed about the status of focal species, aiding proper management decisions in the long-term. Promoting accumulation of long-term data, such as those of Massasaugas in Killbear, is essential for future research.

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Table 2.1: Life history parameters for the Massasauga Rattlesnake (*Sistrurus catenatus*) used in PVA (Vortex) of the Killbear Provincial Park, Ontario population.

Parameter	Values
Adult Initial population	72
Juvenile Initial population	192
Density dependence	None
Carrying Capacity	10 000 (ceiling)
Breeding System	Polygynous
Adult males in breeding pool	100%
Sex Ratio	1:1
Litter Size	10.7
Breeding frequency	Biennial
Age of first reproduction	5 (First year mating) 6 (First litter)
Maximum age of reproduction	12
Maximum lifespan	12
Quasi extinction threshold	10
Density dependent reproduction?	No
Neonate (0-1) Mortality	65%
Juvenile 1 (1-2) Mortality	30%
Juvenile 2 (2-3) Mortality	10%
Juvenile 3 (3-4) Mortality	10%
Juvenile 4 (4-5) Mortality	10%
Adult 1 (5+) Mortality	28%

Table 2.2: QAICc values of POPAN population models in MARK of Massasauga (*Sistrurus catenatus*) Population A, Killbear Provincial Park, Ontario.

Model	QAICc Value
Constant Survival/Recapture	147.7
Constant Recapture	151.1
Constant Survival	154.5
Fully Time Dependent	159.1
Constant Survival/Probability of entry	31478.4
Constant Survival/Recapture/Probability of entry	31840.0

Table 2.3: Population growth rates of Massasauga (*Sistrurus catenatus*) Population A, Killbear Provincial Park, Ontario with observed road mortality rates of each sex.

Male Mortalities	Female Mortalities	Mean Exponential Growth Rate (r) Adults	Mean Exponential Growth Rate (r) Juveniles
5	0	0.011	0.012
4	1	-0.007	0.005
3	2	-0.044	-0.007
2	3	-0.084	-0.025
1	4	-0.117	-0.049
0	5	-0.146	-0.064

Table 2.4: Population growth rates of Massasauga (*Sistrurus catenatus*) Population A, Killbear Provincial Park, Ontario with predicted road mortality rates had mitigation not taken place. Predicted road mortality was set as the post-mitigation mortality (N=5), plus the potential number of Massasauga road mortalities had mitigation not been constructed (number of snakes that crossed ecopassages in 2014).

Male Mortalities	Female Mortalities	Mean Exponential Growth Rate (r)-Adults	Mean Exponential Growth Rate (r)-Juveniles
14	0	-0.196	0.010
13	1	-0.183	0.002
12	2	-0.171	-0.013
11	3	-0.172	-0.033
10	4	-0.177	-0.052
9	5	-0.188	-0.067
8	6	-0.201	-0.081
7	7	-0.218	-0.093
6	8	-0.243	-0.107
5	9	-0.263	-0.118
4	10	-0.278	-0.130
3	11	-0.290	-0.137
2	12	-0.304	-0.143
1	13	-0.312	-0.149
0	14	-0.314	-0.149

Table 2.5: Population growth rates of Massasauga (*Sistrurus catenatus*) Population A, Killbear Provincial Park, Ontario with a low prediction of road mortality rates had mitigation not taken place.

Male Mortalities	Female Mortalities	Mean Exponential Growth Rate (r)-Adults	Mean Exponential Growth Rate (r)-Juveniles
10	0	-0.046	-0.011
9	1	-0.050	-0.003
8	2	-0.079	-0.012
7	3	-0.105	-0.027
6	4	-0.132	-0.045
5	5	-0.157	-0.064
4	6	-0.183	-0.078
3	7	-0.208	-0.094
2	8	-0.233	-0.105
1	9	-0.248	-0.114
0	10	-0.257	-0.122

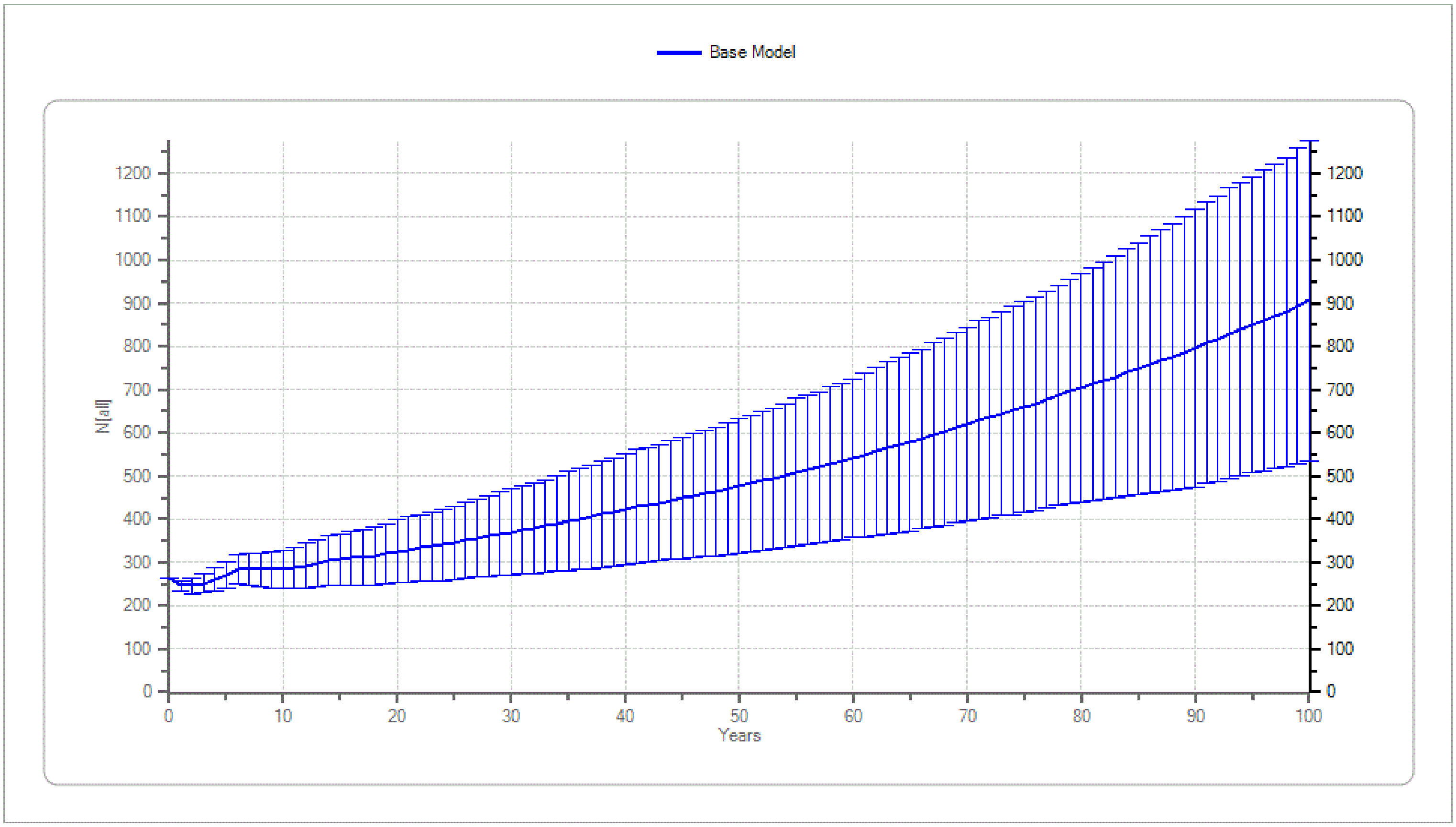

Figure 2.1: Projected mean exponential population growth (500 simulations) of Massasauga (*Sistrurus catenatus*) Population A, Killbear Provincial Park, Ontario. Base model of Massasauga life history parameters without addition of anthropogenic mortality events (road mortality).

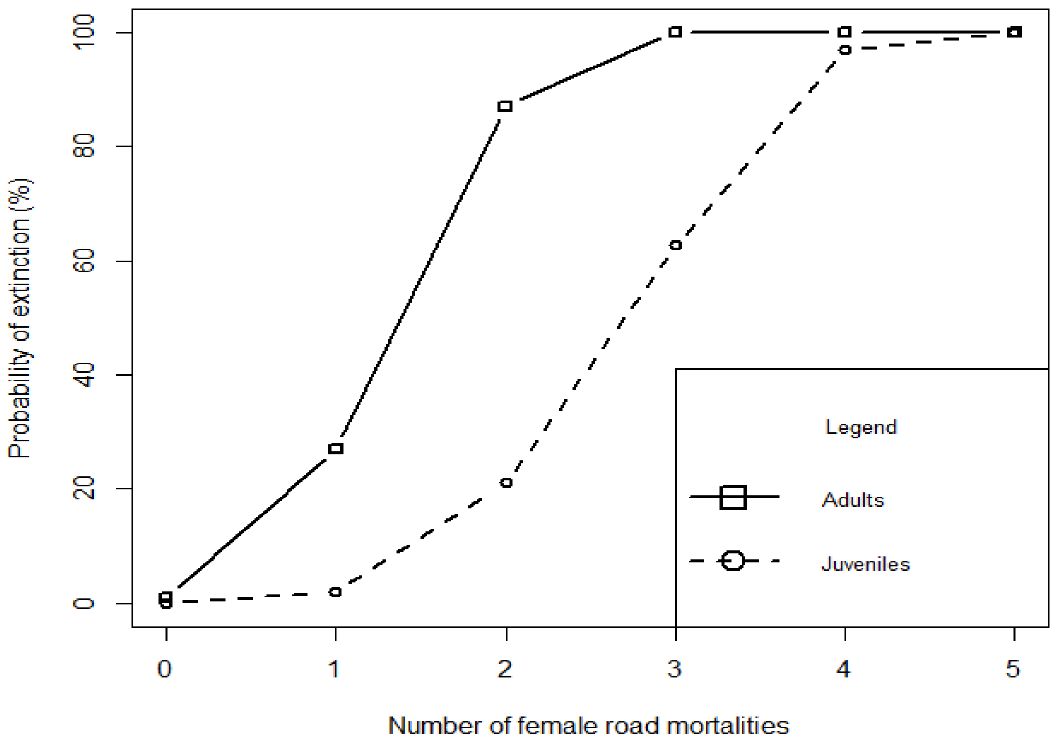

Figure 2.2: Probability of extinction of Massasauga (*Sistrurus catenatus*) Population A, Killbear Provincial Park, Ontario given post-mitigation road mortality rate (N=5). Road mortality was held constant at 5 individuals. Female road mortality varied between 0-5 while male mortality accounted for the remaining individuals.

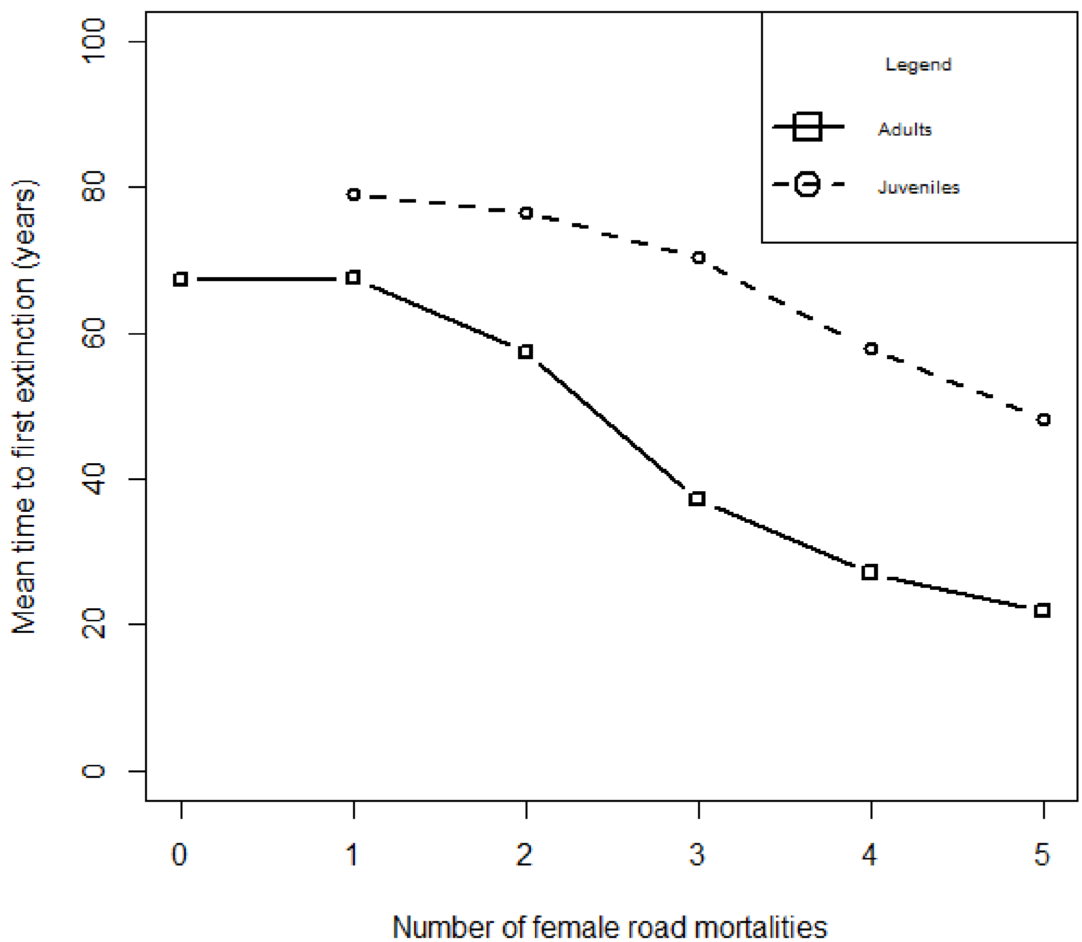

Figure 2.3: Mean time to extinction of Massasauga (*Sistrurus catenatus*) Population A, Killbear Provincial Park, Ontario given post-mitigation road mortality rate (N=5). Road mortality was held constant at 5 individuals. Female road mortality varied between 0-5 while male mortality accounted for the remaining individuals.

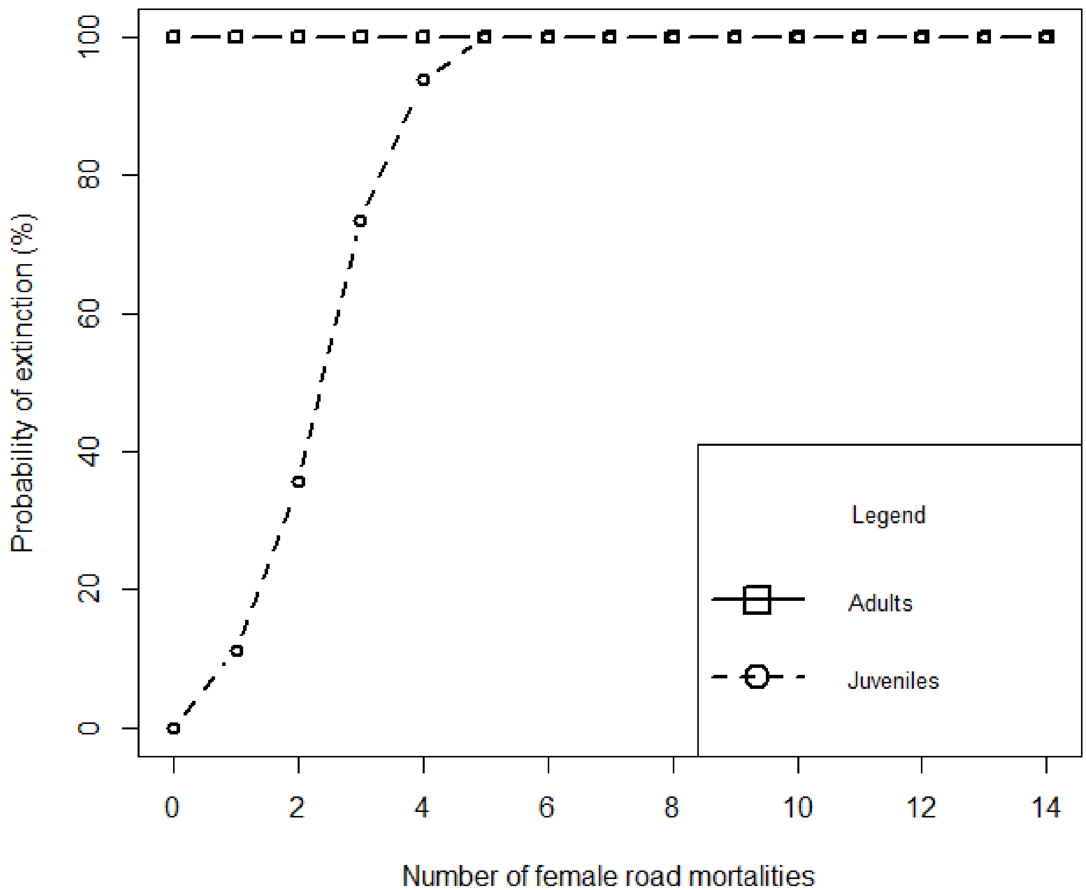

Figure 2.4: Probability of extinction of Massasauga (*Sistrurus catenatus*) Population A, Killbear Provincial Park, Ontario with predicted road mortality rates had mitigation not taken place. Predicted road mortality was set as the observed post-mitigation mortality (N=5) and the potential number of Massasauga road mortalities had mitigation not been constructed (N=9, number of snakes that crossed ecopassages). Road mortality was held constant at 14 individuals. Female road mortality varied between 0-14 while male mortality accounted for the remaining individuals.

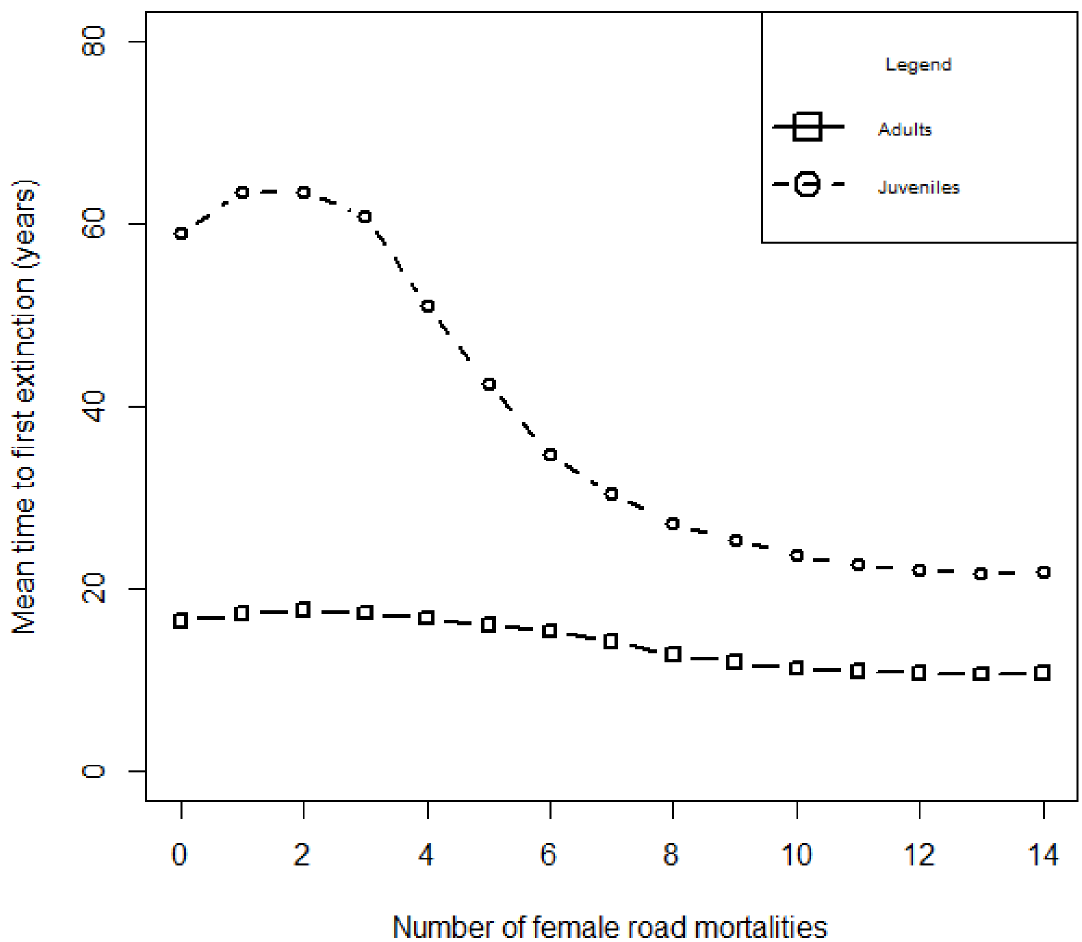

Figure 2.5: Mean time to extinction of Massasauga (*Sistrurus catenatus*) Population A, Killbear Provincial Park, Ontario using road mortality rate (N=14) had mitigation not been constructed. Road mortality was held constant at 14 individuals. Female road mortality varied between 0-14 while male mortality accounted for the remaining individuals.

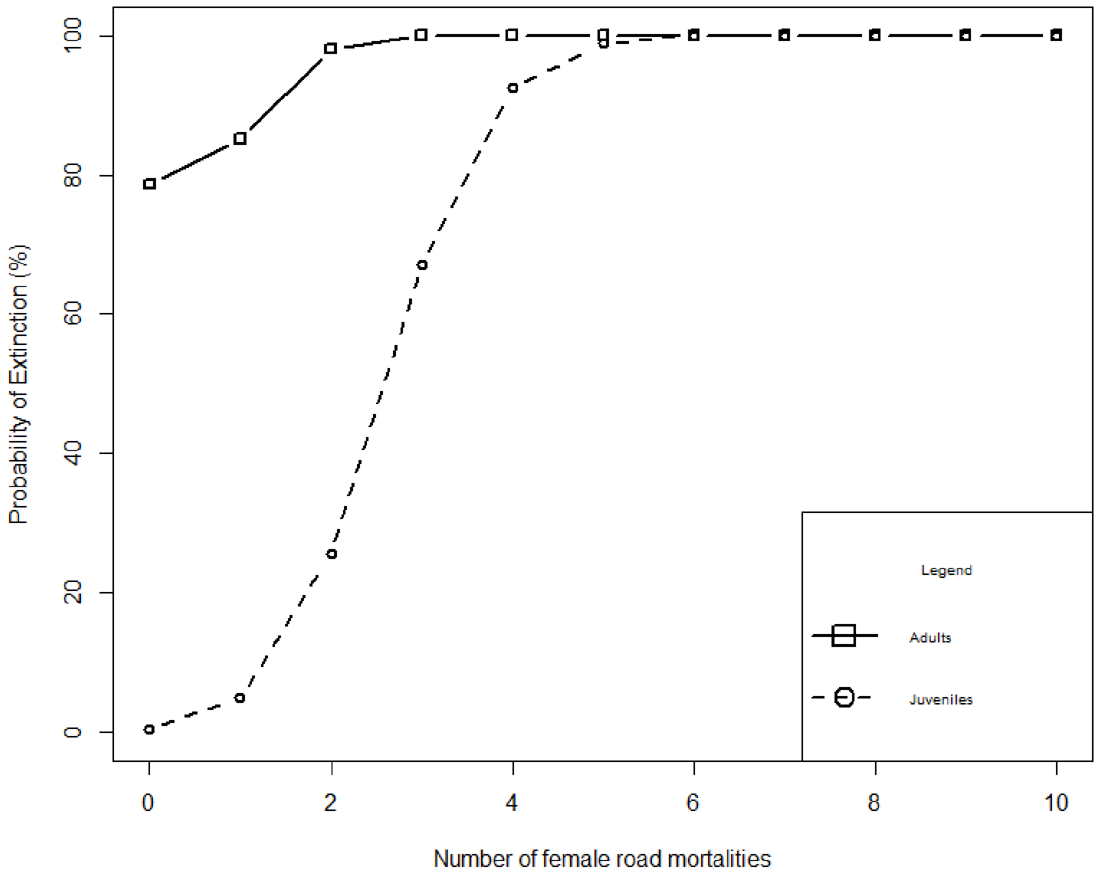

Figure 2.6: Probability of extinction of Massasauga (*Sistrurus catenatus*) Population A, Killbear Provincial Park, Ontario with low predicted road mortality rates had mitigation not taken place. Road mortality was held constant at 10 individuals. Female road mortality varied between 0-10 while male mortality accounted for the remaining individuals.

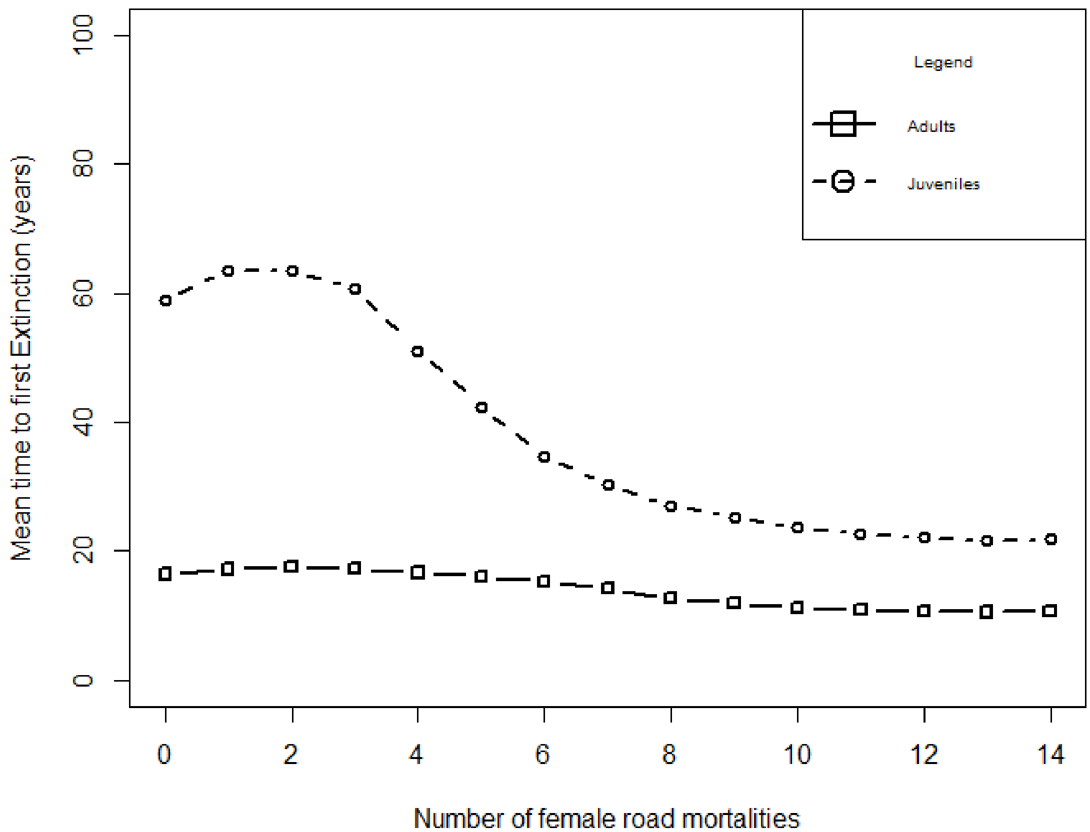

Figure 2.7: Mean time to extinction of Massasauga (*Sistrurus catenatus*) Population A, Killbear Provincial Park, Ontario using low road mortality rate (N=10) had mitigation not been constructed. Road mortality was held constant at 10 individuals. Female road mortality varied between 0-10 while male mortality accounted for the remaining individuals.

General Conclusion

As Ontario continues to experience widespread reptile declines, it is more important than ever to ensure effective road mitigation measures are implemented to protect at-risk reptile species. Road mortality is one of the main factors causing the decline of the Massasauga. Killbear Provincial Park is one of the busiest parks in the province with over 250 000 visitors a year, resulting in significant road mortalities of wildlife, including the Massasauga rattlesnake. Killbear took action to protect the Massasauga by constructing barrier fencing and ecopassages along busy park roads. After mitigation was constructed, Massasauga road mortality has been reduced and bisected habitat has been connected, resulting in an apparently reduced extinction risk of this population. Although successful, below I outline several recommendations to enhance the success of the mitigation measures and promote a sustainable population of Massasaugas into the future.

1) **Permanent fencing:** To avoid costly and labour intensive annual repair, a permanent form of fencing should be installed. Minor changes in fencing placement should be planned to avoid inundated areas that damage fencing over time. Fencing material should be selected to prevent smaller-bodied snakes and turtles from accessing the road, unlike the current fence.
2) **Maintain ecopassages:** Snakes are willing to use the current ecopassages and therefore their design is effective so long as it is maintained. Debris should be removed from inside of the ecopassages annually, as substrate builds up and reduces access to and thus efficacy of ecopassages.

3) **Additional mitigation:** The current fencing situation is allowing for Massasaugas to persist in Killbear, but this should be closely monitored for changes in movement patterns over time. Mortality of Massasaugas and other reptile species still occurs in the park. Therefore if possible, additional fencing should be installed along the hot spot on the main park road that I identified. This fencing should prevent small-bodied snakes (and amphibians) from accessing the road. In addition, an ecopassage should replace the current structural culvert on the main park road beside the Blind Bay parking lot to facilitate snake, turtle and amphibian crossings.

4) **Promote awareness:** Killbear should work with Carling Township and local cottagers to raise awareness of snake mortality hot spots along cottage roads. Signage should be installed on the stretch of road that encompasses the hot spots, suggesting that drivers slow down and be vigilant for snakes crossing these sections of the road.

5) **Future research priority-Population B:** A full analysis of Population B was not completed in my study due to insufficient data. Given the lack of recent Massasauga captures and historically-high road mortality of Massasaugas in Population B, this subpopulation needs immediate attention to determine its current status.

6) **Continue data collection:** The Killbear long-term database has proved valuable for assessing the effectiveness of mitigation strategies. If financially feasible, Killbear staff should continue to PIT-tag Massasaugas and contribute to the long term database to ensure research can be conducted well into the

future. The monitoring of ecopassages via automated PIT-tag readers should continue. The PVA should be updated as new data are collected or the current situation changes (road mortality rates, mass mortality events, etc.) to ensure the most accurate representation of this population's viability.

A key facet of my work is that it would not have been completed without the full cooperation from all stakeholders and landowners. Ensuring that all parties are engaged in or informed of various initiatives for species protection helps to ensure that appropriate action is taken to conserve species for future generations. Projects such as mine prompt the curiosity of the public to enquire into the protection and preservation of Ontario reptiles and help inform important stakeholders and the public of the negative effects that roads have on wildlife.